VOLUME 60 • OCTOBER 1958 • NUMBER 4

OPHTHALMOLOGY

PART I

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W. Morton Grant

The Rule of Seven or Fourteen Thirds

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A Simplified Method of Stereophotography of the Eye

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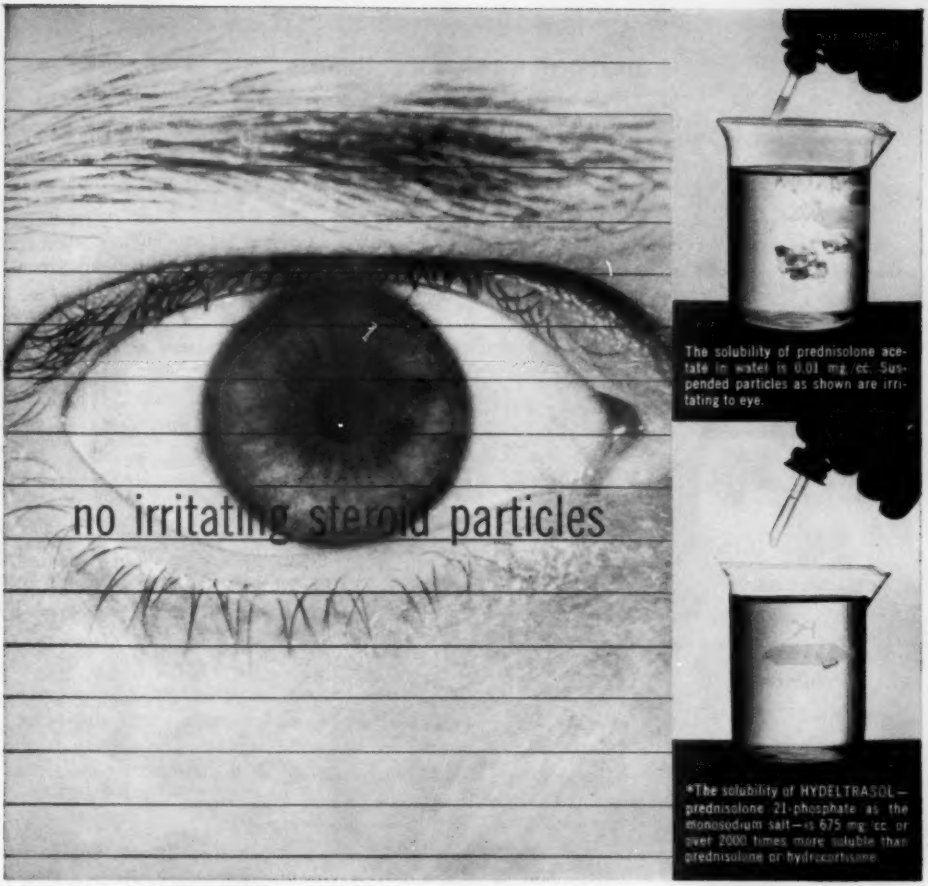
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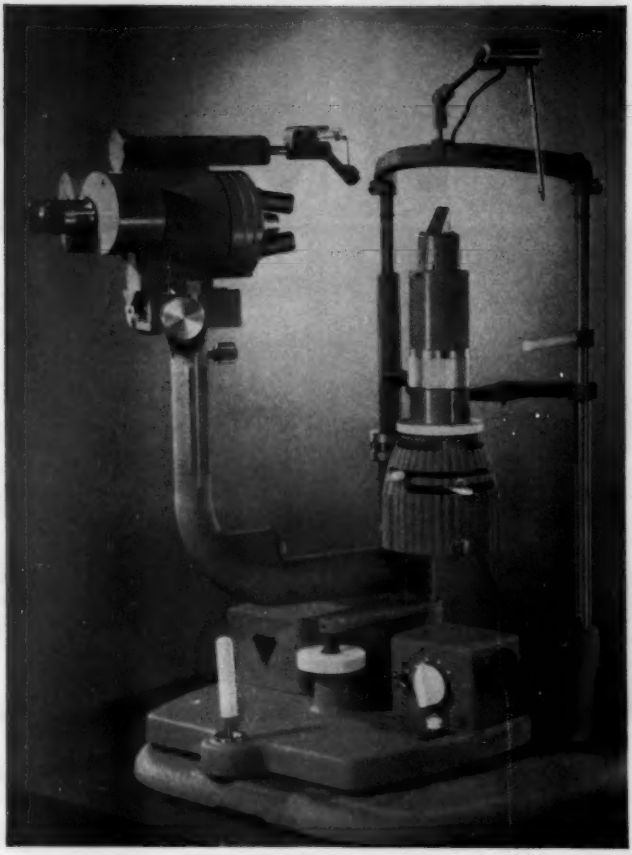

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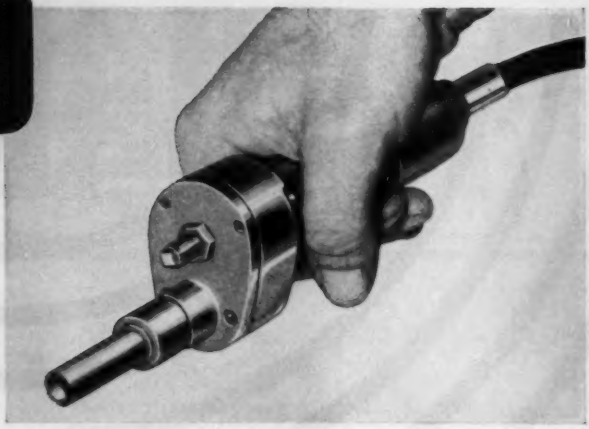

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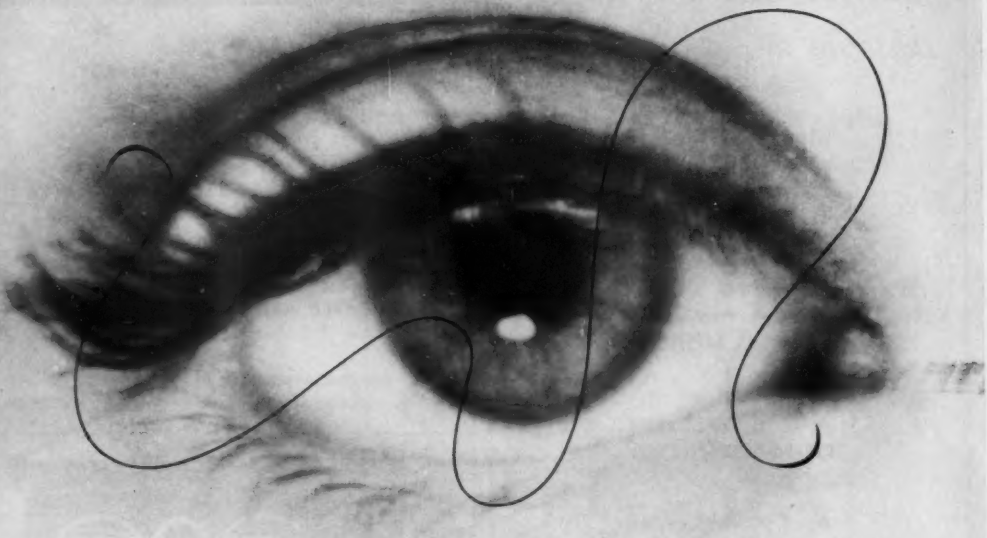

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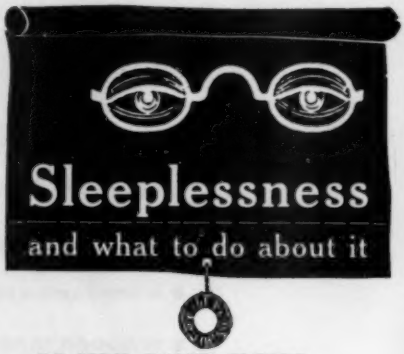

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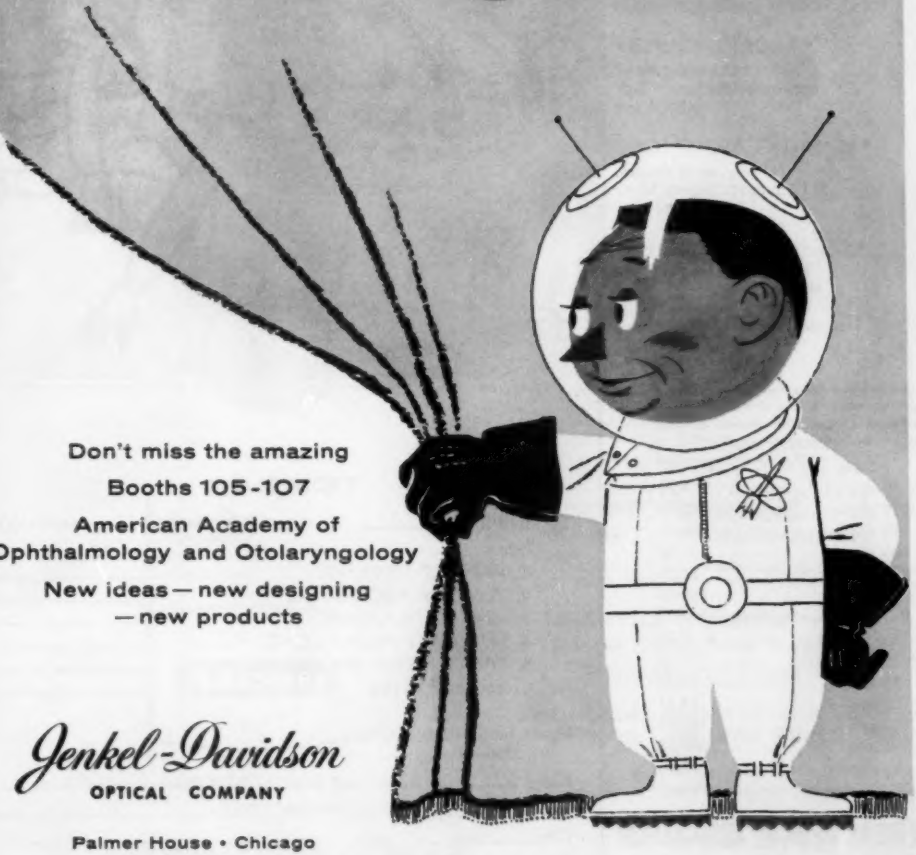

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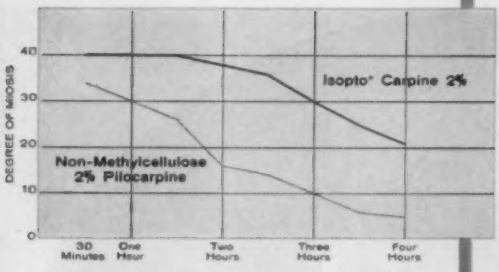

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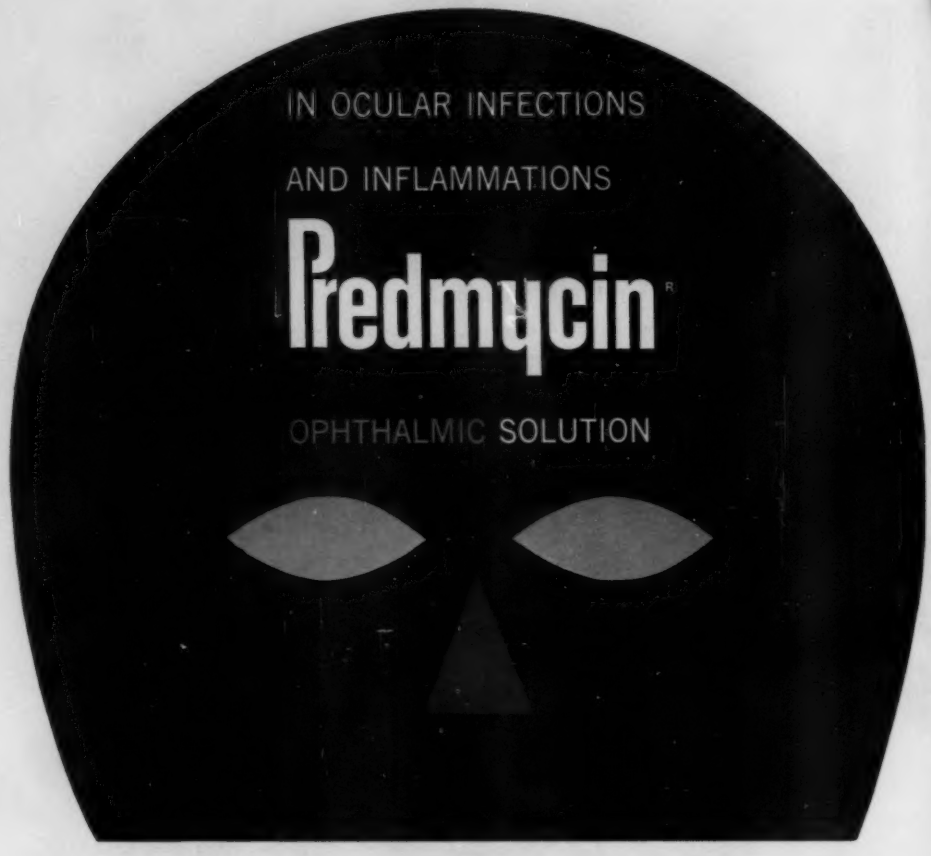

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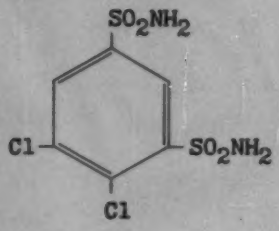

MODE OF ACTION

'Daranide' is primarily a carbonic anhydrase inhibitor; i.e., it interferes with the catalytic activity of this enzyme, which is concerned with the formation of H^+ and HCO_3^- ions and the conservation of base as sodium bicarbonate. Under the influence of 'Daranide' the renal excretion of sodium, potassium, and bicarbonate is increased and an alkaline urine is produced. However, this compound also has the

advantage of causing a definite increase in chloride excretion. Partially as a result of the greater excretion of chloride, 'Daranide' does not produce metabolic acidosis as readily as other compounds of this nature. Furthermore, in animal studies 'Daranide' has been shown to maintain its essential activity over the course of weeks or months.

The compound does not appear to be concentrated in gastric mucosa in sufficient amounts to influence materially the activity of carbonic anhydrase in this tissue. Thus, in dogs, gastric motility or secretion is not affected to any significant degree by the administration of 'Daranide'.

PHARMACOLOGY

'Daranide' is uniformly and well absorbed following its oral administration; it is essentially as active when given orally as parenterally. The enhancement of sodium, potassium, and chloride excretion by the oral administration of a single dose is rapidly manifested and persists for a number of hours, indicating a prolonged duration of action of the compound. The urinary pH is elevated as the result of sustained bicarbonate excretion. However, greater chloruresis and less acidosis are induced than with other carbonic anhydrase inhibitors, with consequent greater efficacy on repeated administration. The natriuretic effect of 'Daranide' has been observed even after 10 weeks of continued administration to dogs. The activity of 'Daranide' is not impaired in the presence of an acid urine such as that accompanying the administration of ammonium chloride.

'Daranide' has a low inherent toxicity when administered orally or parenterally to laboratory animals. The compound has been given orally to dogs and rats over a period of at

least six months with essentially no evidence of toxicity. Pharmacologic investigation indicates an absence of irritating effect on the mucosa of the eye as demonstrated by a lack of local anesthetic activity or irritation when a 1 per cent concentration of the drug (suspended in tragacanth) is instilled into the conjunctival sac of the rabbit eye.

CLINICAL EVALUATION

Effect on Intraocular Tension

Studies in humans have demonstrated that 'Daranide' administered orally reduces intraocular pressure in both glaucomatous and nonglaucomatous eyes. The onset of action following a single dose is within an hour and maximal effect is observed within two to four hours. The lowered intraocular tension is maintained for approximately six hours. It has been possible to control excessive intraocular pressure in patients with glaucoma for a period of many months by the daily combined use of 'Daranide' and miotics. Tonographic studies have shown that 'Daranide' lowers intraocular pressure by inhibiting the formation of aqueous humor in normal as well as in glaucomatous eyes.

Effect on Electrolytes

In the human as in the laboratory animal, 'Daranide' enhances the excretion of sodium, potassium, bicarbonate, and water. An increased chloride excretion greater than that following the use of other carbonic anhydrase inhibitors is also observed with 'Daranide'. This enhanced excretion of chloride explains, at least in part, the continued efficacy of 'Daranide' with repeated administration and the lesser propensity for the development of metabolic acidosis.

As is the case with all carbonic anhydrase inhibitors, 'Daranide' in high doses causes some

decrease in renal blood flow and glomerular filtration rate.

INDICATIONS

'Daranide' is indicated for oral treatment of various types of glaucoma:

chronic simple (wide angle) glaucoma
acute congestive (narrow angle) glaucoma
chronic congestive glaucoma
secondary glaucoma (acute phase)
preoperative control of intraocular tension of glaucoma

DOSAGE

The usual adult dosage range is from 25 to 50 mg. (½ to 1 tablet) once to three or four times daily. Dosage must be carefully adjusted to meet the requirements of the individual patient.

Therapy with 'Daranide' is usually most successful when employed in conjunction with miotics such as pilocarpine, physostigmine, 'Floropryl' (isoflurophate), or 'Carcholin' (carbachol). In acute congestive glaucoma, 'Daranide,' with miotics, should be administered promptly in an attempt to bring about rapid reduction of intraocular tension. If this is not quickly relieved, surgery may be mandatory. In chronic congestive glaucoma, surgery may be necessary if the intraocular tension is not brought successfully under control after an adequate trial period of therapy with 'Daranide'.

PRECAUTIONS

Since 'Daranide' is a carbonic anhydrase inhibitor, certain side effects characteristic of this class of compounds will occur, particularly in the higher dosage ranges. These effects may in-

clude anorexia, nausea, vomiting, nervousness, lassitude, depression, confusion, disorientation, sedation, dizziness, ataxia, tremor, tinnitus, paresthesias, and pruritus. Fortunately, the therapeutic amount of 'Daranide' required for most patients is well beneath the level that will produce these effects. When they do occur, reduction of dosage or temporary discontinuance of the drug is indicated.

It is essential to keep a close check on the electrolyte balance of the patient during therapy with 'Daranide', as with patients on any other carbonic anhydrase inhibitor.

SUPPLIED

'Daranide' Tablets, 50 mg., bottles of 100 and 1000.

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1. Schlegel, H. E., Jr., and Swan, K. C.: A.M.A. Arch. Ophth. *51*:663 (May) 1954.
2. Emmerich, R.; Carter, G. Z., and Berens, C.: Am. J. Ophth. *40*:841 (Dec.) 1955.

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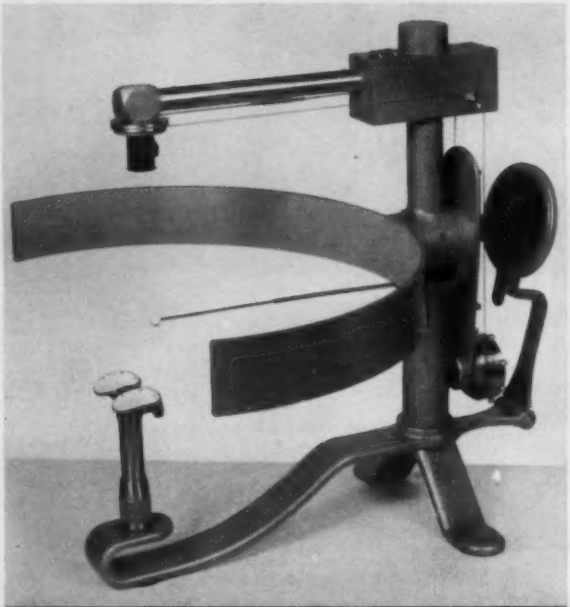

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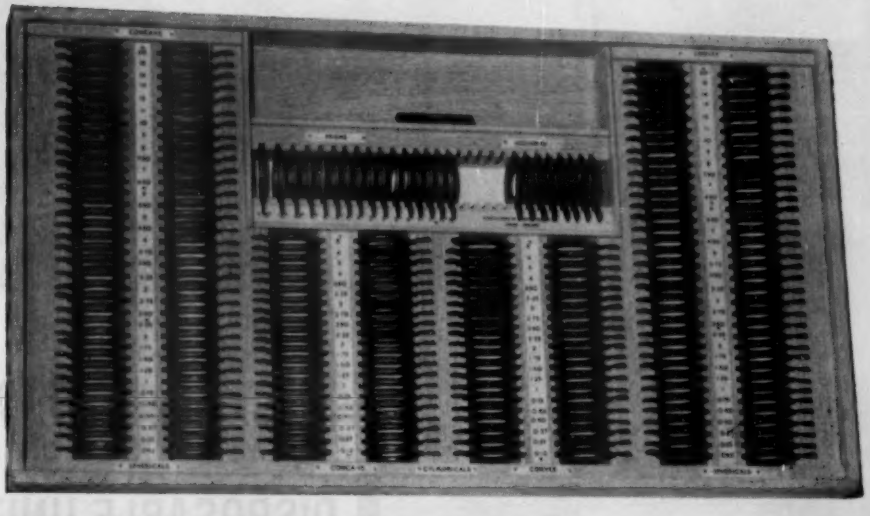

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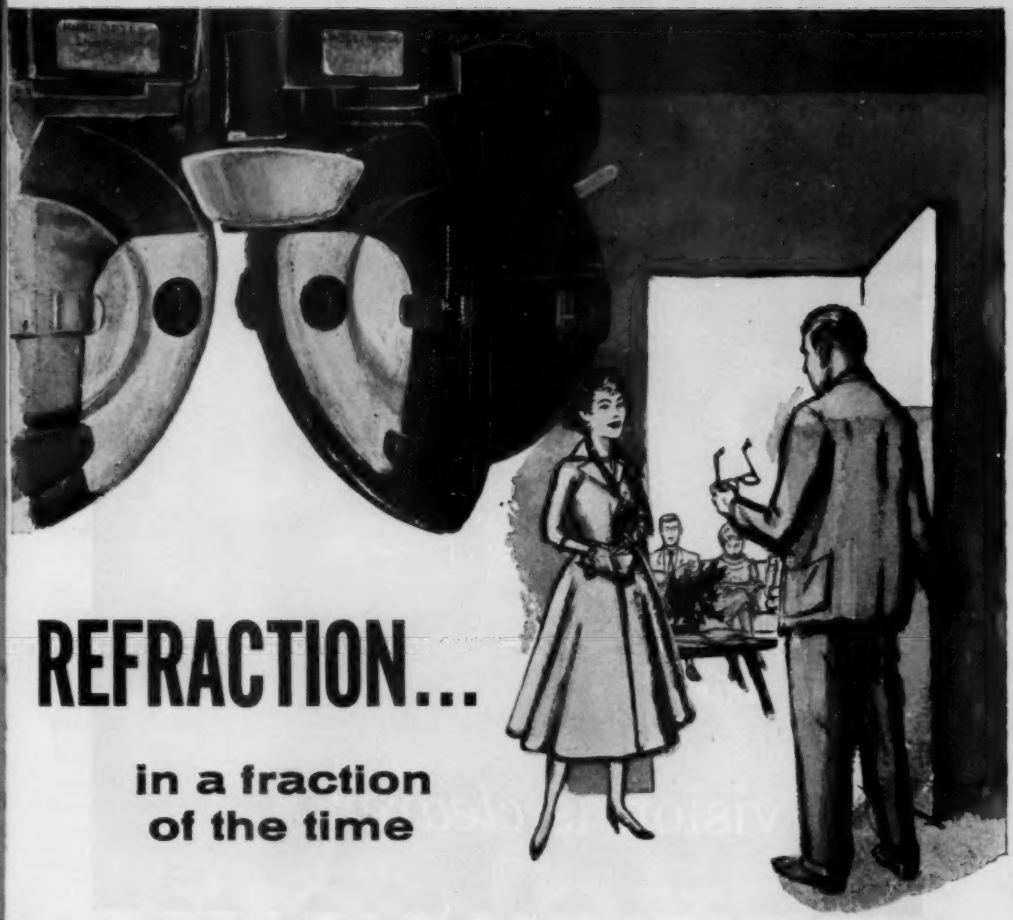

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Further Studies on Facility of Flow Through the Trabecular Meshwork

W. MORTON GRANT, M.D., Boston

In 1954 and 1955 preliminary results were reported on attempts at measuring directly how much of the normal resistance to outflow of aqueous humor is attributable to the trabecular meshwork in human eyes.[1,2] In the more recent of these experiments enucleated human eyes were perfused with sodium chloride solution through a needle which entered the anterior chamber through the cornea and communicated with both anterior and posterior chambers. The facility of outflow was determined by measuring both the rate of flow and the intraocular pressure after a steady state was attained. Then, the trabecular meshwork in one quadrant was curetted with the point of the transcorneal needle, and the facility of outflow was redetermined. In experiments on 19 eyes, no change in facility of outflow was found in 12, but an increase occurred in 6, and a small decrease, in only 1. Microscopic examination of sections of all eyes indicated that the trabecular meshwork had been torn open apparently into Schlemm's canal in each instance, but there was also considerable damage to adjacent sclera. At that time I could not recognize any significant difference microscopically between eyes having a change in facility of outflow and those having no change. My tentative interpretation was that the trabecular meshwork offered little resistance to flow, since it could be curetted with no change in facility of outflow in the majority of instances; in those cases in which facility of outflow was increased I thought it possible that the ostia of collector channels had been torn open. The results of these experiments were, obviously, far from conclusive, and the possible and probable shortcomings were discussed at some length at the Macy Foundation Conference on Glaucoma of 1955.[3] At the same conference Bárány presented evidence of considerable resistance to flow in structures of the chicken eye analogous to the trabecular meshwork of the human. On the other hand, Perkins has reported that when he cannulated Schlemm's canal in monkey eyes he found very little difference between the pressure in this canal and the pressure in the anterior chamber, suggesting that the aqueous humor encounters only slight resistance to flow through the trabecular meshwork.[4]

I have been dissatisfied with my own previously reported experiments, particu-

Submitted for publication Dec. 16, 1957.

Howe Laboratory of Ophthalmology, Massachusetts Eye and Ear Infirmary, and Harvard University Medical School.

This investigation was supported by the Alfred P. Sloan Foundation and by Research Grant B-218 of the Institute of Neurological Diseases and Blindness of the National Institutes of Health, U. S. Public Health Service.

Normal enucleated eyes were supplied for this study by the Boston Eye Bank. Joann S. Perkins prepared fixed tissues for histologic study. The special stainless steel fitting was made to my specifications by Werner Mueller, instrument maker at the Howe Laboratory.

larly because of the essentially blind and poorly controlled manner in which the trabeculotomies were performed. The technique has now been improved so that removal of the trabecular meshwork is performed under direct microscopic observation in order to render the operation more precise and to minimize chance of injury to deeper structures, such as the collector channels. With this technique I have obtained new and more consistent findings which indicate that the trabecular meshwork does in fact present the principal resistance to aqueous outflow.

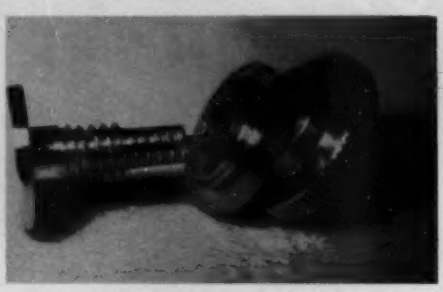

Fig. 1.—This stainless steel fitting is employed to close a hole in the cornea, permitting measurement of facility of outflow by perfusion via the tube in the center of the fitting; when the steel fitting is removed, the hole in the cornea provides access for dissection of the trabecular meshwork under microscopic observation.

Experimental Procedure

An important part of the improvement in the technique for these experiments has been achieved by invention of a means for opening and tightly closing a hole in the cornea through which to dissect the trabecular meshwork under direct view with a microscope and to make measurements of facility of outflow by perfusion. The new arrangement provides a much better view of the angle of the anterior chamber than is generally possible through the corneas of enucleated eyes and permits effective use of magnification of 40 times or more. At the same time, it obviates the distortion of the cornea which is caused when an instrument is engaged tightly in the cornea itself, and it permits use of various types of instruments other than those which can be inserted in a self-sealing manner through the cornea. It also makes the whole circumference accessible for operation; in the more customary method for goniotomy, in which the cornea is pierced by an instrument near the limbus, it is not feasible to reach much more than a third of the cir-

Fig. 2.—The stainless steel fitting is installed in a hole trephined in the cornea; the flanges clamp the cornea at the edges of the hole to make a tight seal; apparatus for measuring intraocular pressure and rate of flow connects to the eye by way of a tube in the center of the fitting.

cumference. In the new arrangement the provision which is made for tight reclosure of the opening in the cornea, and an additional provision which is made for connecting a perfusion tube to the eye, are essential for determining the facility of outflow of intraocular fluid by the perfusion method.

The desired opening in the cornea through which to view with a microscope and to operate has been made by means of a 5 mm. corneal trephine, and the necessary tight reclosure of the opening has been secured by means of a special stainless steel fitting. The device employed, shown in Figures 1 and 2, is comprised of three parts: a disc of 6 mm. diameter permanently attached to the end of a hollow threaded rod; a flat stepped annulus sliding over the threaded hollow rod, having a diameter of 6 mm. at its broader face and 5 mm. diameter at its smaller face, and a knurled nut to tighten the two other parts together. In use, the disc at the end of the hollow rod slips through the 5 mm. opening in the cornea in the manner that a button goes through a buttonhole. The flat annulus comes against the cornea on the outside and is centered automatically by the smaller stepped portion which fits accurately into the 5 mm. hole in the cornea. When the knurled nut is tightened, the cornea is clamped in a secure and leakproof manner, acting itself as a gasket. The opposed clamping surfaces are made slightly concave so that the tightest seal is at the outer edges, and there is no tendency for the cornea to be squeezed out of the clamp. The fitting installed in the cornea is inspected frequently with a binocular microscope each time it is used to be sure there is no leak. For perfusion measurements, connection to the anterior chamber is made by way of the hollow center of the threaded rod. A stainless steel tube of smaller diameter is soldered into the outer end of this rod for convenience in making connection with fine flexible plastic tubing to apparatus for measuring pressure and flow rate.

For viewing the structures of the angle of the anterior chamber through the axial hole in the cornea, the eye, with the steel fitting removed, was held with its axis essentially vertical, and the amount of fluid in the anterior chamber was adjusted so that it formed a meniscus with concave surface in the opening. (A convex or flat surface did not afford a satisfactory view.) Alternatively, the fluid in the anterior chamber could be replaced with air to give a direct view of the angle. However, keeping saline in the anterior chamber during observation and dissection had the advantage that it avoided effects of surface tension in the angle at the interface of air and liquid; in the presence of air the wet tissue surfaces tend to cling together, sometimes causing the iris to stick to the back of the cornea in inconvenient manner. Dissection of the trabecular meshwork has been carried out under direct view with a slit-lamp biomicroscope or with a binocular dissecting microscope having nearly coaxial illumination. In most instances a magnification of 40 times has been utilized. The 5 mm. hole in the cornea provides sufficient room for illumination, observation, and surgical instrumentation at the same time.

The experimental procedure employed in the present studies has been first to trephine out the 5 mm. disc of cornea, install the stainless-steel fitting, and make a measurement of the facility of outflow by determining the relation of flow rate to intraocular pressure under steady-state conditions by a technique previously reported, employing 0.9% sodium chloride solution for perfusion.[1,2,5] The fitting has then been removed from the cornea and operation has been performed on the trabecular meshwork under direct view with the microscope. The corneal fitting has then been reinstalled, and the facility of outflow, redetermined. (In control experiments inserting and removing the corneal fitting without operation on the meshwork had no appreciable influence on facility of outflow.) The corneal fitting has been removed and replaced without difficulty several times in individual

eyes when the influence of successive stages of surgery on the meshwork was to be evaluated. Lastly, all eyes were fixed in formaldehyde solution. In occasional eyes either colored latex or India ink was injected into the anterior chamber before fixation. After fixation the eyes were dehydrated, embedded in nitrocellulose, and sectioned.

Operating with a direct magnified view of the trabecular meshwork has made possible a considerable refinement in the technique employed for trabeculotomy. Where previously the procedure consisted of plowing up the meshwork and surrounding tissues by curetting or scraping with the broadest aspect of the point of a 20-gauge intravenous needle, without adequate view of the site of operation, the aim in the present experiments has been to open or remove the meshwork as precisely as direct inspection would make possible and at the same time to avoid injury to the scleral wall of Schlemm's canal. This has been attempted variously as follows. In three eyes the meshwork was torn open by introducing the tip of one blade of a fine-pointed toothless forceps into Schlemm's canal with the rounded outer aspect of the blade against the scleral wall. The tip was moved circumferentially, tearing apart the trabeculae as it went. In one of these eyes an unsuccessful attempt had been made previously to remove the meshwork by suction applied through a fine glass tube having a diameter almost equal to the distance from scleral spur to Schwalbe's line. In six eyes the trabecular meshwork was opened by means of a von Graefe cystotome introduced across the anterior chamber with the shank not quite perpendicular to the meshwork. The tip of the cystotome was introduced through the meshwork and into Schlemm's canal so that its blunt outer aspect was against the scleral wall of the canal. The tip was then moved circumferentially in such a manner that the trabecular meshwork rode up on the sharp inner edge and was cut open. In seven eyes the meshwork was incised with a Barkan goniotomy knife in various ways, and at various locations, with an aim of determining how surgery on the trabecular meshwork might be carried out more effectively on glaucomatous patients. The influences of these various procedures on facility of outflow are described in the section on results.

A noteworthy incidental precaution taken in both the present and the previous series of experiments is as follows. In all experiments designed to evaluate how much resistance the trabecular meshwork presents to outflow of aqueous humor it is necessary to control the amount of tension applied by the ciliary muscle to the scleral spur and trabecular meshwork, because this tension exerts a considerable influence on facility of outflow. In enucleated eyes the ciliary muscle itself is usually not actively contractile, but tension is transmissable through the passive muscle from the lens-iris diaphragm when this diaphragm is forced from its normal position, e. g., by artificial deepening of the anterior chamber. In the experiments of the series to be reported in this paper the tension which might come from differences in filling of posterior and anterior chambers was eliminated by providing direct two-way communication between the chambers by placing a small piece of polyethylene tubing under the pupillary border of the iris or by performing peripheral iridectomy. Under these conditions the anterior chamber remained at a constant normal depth during all measurements of facility of outflow. It is believed that the relaxed or neutral state of the lens-iris diaphragm and ciliary muscle under these conditions resembles the state in the living eye in the absence of physiologically or pharmacologically induced accommodation and miosis. The facility of outflow determined under these conditions by perfusion in enucleated eyes comes close to the value obtained by tonography in unstimulated normal eyes of patients.

Results

During the procedure of opening the trabecular meshwork by the different methods employed in the present study the following effects were observed with the microscope. It was found that the meshwork could be torn in small areas by grasping and pulling this tissue with toothless forceps, but more extensive opening was achieved when one tip of a fine curved forceps blade was introduced through a small hole which had been made in the meshwork and, with the blade directed along Schlemm's canal, the tip was moved circumferentially in the sulcus between scleral spur and sclera, i. e., in Schlemm's canal. This procedure caused the meshwork to part at about its midportion, leaving a fringe attached anteriorly to Schwalbe's line. At the same time, this procedure caused variable tearing of the meshwork away from the scleral spur, carrying with it the uveal meshwork (overlying the ciliary muscle between iris periphery and scleral spur) as well as some of the fibers of the ciliary muscle itself. The result was that anterior to the scleral spur a glistening white sulcus of the opened-up Schlemm's canal was evident, with an occasional tiny slit-like opening in the scleral wall (thought to be ostia of collector channels), while posterior to the scleral spur the gray ciliary muscle was laid bare. In one eye the ciliary muscle became completely detached from the scleral spur, leaving sclera exposed. (Incidentally, in a series of experiments to be reported elsewhere I have found that cyclodialysis without opening the trabecular meshwork does not appreciably increase facility of aqueous outflow in enucleated human eyes.) The conclusion was reached that tearing the meshwork with a noncutting instrument was effective in opening this tissue widely but that by this technique the tearing could not be restricted to this tissue alone.

In the group of eyes in which the trabecular meshwork was opened by means of a von Graefe cystotome the effect was found to be much better limited to the desired site. In this series, as the tip of the cystotome passed along circumferentially in Schlemm's canal and the trabeculae rode up on the cutting edge they were seen to be severed cleanly. The cutting occurred at about midzone of the meshwork or slightly closer to the scleral spur. The tissue itself seemed to have little or no tendency to retract, for when it was incised in only an hour or two of the circumference the edges appeared to fall back in apposition. However, when the incision was extended circumferentially for a greater distance or when the scleral spur or the attached ciliary muscle was pressed slightly posteriorward with the side of the blade, the cut edges gaped and gave a view of a glistening white sulcus just anterior to the scleral spur. The spur itself provided a rather resilient posterior lip to the opening. No cyclodialysis was produced when a sharp-edged cystotome was employed.

In the group of eyes in which the trabecular meshwork was incised with the tip of a Barkan goniotomy knife various results were produced, depending upon the site and manner of incision. Cutting circumferentially at the anterior edge of the meshwork close to Schwalbe's line did not cause the meshwork to separate from the sclera, nor did it cause the edges to evert. However, it was apparent from observation of movement of pigment particles enmeshed in the midzone of the trabecular meshwork that the knife caused some traction and dragging of fibers in this adjacent portion of the tissue as well as the intended cutting of the anterior edge. It was found that after incision of the anterior edge the whole width of the meshwork could be pulled away from the sclera and folded toward the iris by drawing the tissue posteriorly with the flat of the knife blade. This more or less bared the white scleral wall down to the scleral spur, but some fine strands of the meshwork remained adherent. When pigment was present within the midzone of the meshwork, this went along with the tissue as it was folded back away from the sclera. One difficulty encountered with this

technique in two eyes was that when the meshwork had been separated from the scleral wall back to the scleral spur it very easily became detached from the spur itself. Since the meshwork and ciliary muscle remained continuous, the detachment from the scleral spur separated the ciliary body as well as the trabecular meshwork from the spur, in effect causing a trabeculocyclodialysis. In eyes in which the initial incision was made into the posterior portion of the meshwork just anterior to the scleral spur, with the tip of the Barkan goniotomy knife held nearly perpendicular to the tissue, there appeared to be no tendency to cause detachment of the ciliary body from the spur. Cutting in this manner gave results similar to those obtained when the meshwork was slit open by means of the cystotome; those results have already been described. Incision in an arc limited to a quadrant or less did not cause the edges to gape spontaneously, although they did gape when a little pressure was exerted with the side of the blade on the resilient posterior lip formed by the scleral spur or on the attached ciliary muscle. There was more tendency for the wound to remain open and reveal the white scleral wall when the tissue was incised over a greater portion of the circumference.

Combined incision of both anterior and posterior edges of trabecular meshwork with the goniotomy knife gave varied results. When the anterior edge was incised first, it was difficult to incise the posterior edge, because, without its normal anterior attachment, the tissue tended to slide and gather ahead of the blade instead of cutting cleanly. When the posterior edge of the meshwork (just anterior to the scleral spur) was incised first, it remained fairly easy subsequently to incise the anterior edge (at Schwalbe's line), and there was relatively little tendency for the loose tissue to gather in front of the blade. When the trabecular meshwork was cut across to interrupt its circumferential continuity in addition to having incisions at its anterior and posterior edges, it became possible to remove the tissue as a whole. This maneuver exposed the scleral wall from Schwalbe's line to scleral spur, revealing above the spur the sulcus which formerly constituted the outer aspect of Schlemm's canal, and left the ciliary body attached to the posterior aspect of the spur.

The observations which have just been described were made in association with measurements of the facility of outflow from the anterior chamber, and in several instances measurements were made at successive stages of the operative procedures. In the series of 16 enucleated normal human eyes upon which the trabeculotomy and trabeculectomy procedures were performed as already described, a significant increase in facility of outflow (decrease in resistance to outflow) was found in every instance. The results for each eye are listed in the Table. The amount of change was approximately related to the proportion of the circumference operated upon,* and calculations from the experimental data indicate that the trabecular meshwork as a whole, in a relaxed state, is responsible for approximately 75% of the total resistance to outflow of aqueous humor from normal human (enucleated) eyes. The remaining 25% of the resistance to outflow is presented by the intrascleral vessels through which the aqueous must pass to reach the surface of the globe. Incidentally, no appreciable change in resistance was found when episcleral tissues were removed, and in an earlier investigation when the outer two-thirds of the sclera was dissected away no significant change in resistance was observed[1]; apparently most of that portion of resistance which is in the intrascleral vessels is within the inner third, i. e., close to Schlemm's canal.

The average value for facility of outflow in the 16 eyes of the present series shown in the Table was 0.147 μl. (microliters) per minute per millimeter of Hg at a room

* On the average, for each tenth of the circumference in which the trabecular meshwork was opened or removed the facility of outflow was found to be increased by 30%.

Effect of Dissection of the Trabecular Meshwork on Facility of Outflow of Intraocular Fluid in Enucleated Normal Human Eyes

Eye No.	Instrument Employed	Nature of Operation on Meshwork	Proportion of Circumference Operated On	Change in Facility of Outflow (at 24 C), μl/Min/Mm. Hg	Calculated Decrease in Resistance,* %	Proportion of Initial Resistance Attributable to the Meshwork, † %
1	Forceps blade	Tearing open	Whole	0.13→0.61	79	79
2	Forceps blade	Tearing open	75%	0.17→0.36	53	
3	Forceps blade	Tearing open	Whole	0.07→0.32	70	70
4	Cystotome	Slitting open	Whole	0.19→0.51	63	63
5	Cystotome	Slitting open	Whole	0.27→1.05	74	74
6	Cystotome	Slitting open	Whole	0.12→0.56	79	79
7	Cystotome	Slitting open	Whole	0.19→0.68	72	72
8	Cystotome	Slitting open	Whole	0.16→0.80	80	80
9	Cystotome	Slitting open	Whole	0.15→0.60	75	75
10	Knife	Incision of posterior meshwork	Whole	0.15→0.60	75	75
11	Knife	Incision of anterior meshwork	Whole	0.16→0.41	61	—
		Incision posteriorly and removal of meshwork	Whole	→0.64	75	75
12	Knife	Incision of posterior meshwork	10%	0.10→0.16	38	—
		Incision of posterior meshwork	50%	→0.30	67	80
13	Knife	Incision of posterior meshwork	15%	0.08→0.16	50	—
		Incision of anterior edge and removal of meshwork	15%	→0.18	55	80
		Incision both posterior and anterior and removal of meshwork	33%	→0.54	85	95
14	Knife	Excision	15%	0.13→0.20	35	88
		Excision	33%	→0.43	70	87
		Anterior incision and detachment of meshwork	70%	→0.68	81	86
15	Knife	Anterior incision and detachment by traction	75%	0.13→0.43	70	75
16	Knife	Anterior incision and detachment by traction	75%	0.15→0.92	84	88

* Decrease in resistance to outflow was calculated in per cent by the formula $100(1-\frac{C_1}{C_2})$.

† The proportion of the whole initial resistance to outflow which may be attributed to the meshwork was calculated in per cent by the formula $100(1-\frac{aC_1}{C_2-(1-a)C_1})$.

The symbols and terms employed in setting up these formulas were as follows:
C_1=facility of outflow determined preoperatively
C_2=facility of outflow determined postoperatively
X=fraction of total resistance to outflow attributable to the meshwork
a=fraction of the circumference operated upon
aC_1=facility of outflow of operated-on portion preoperatively
$(1-a)C_1$=facility of outflow of intact portion both pre- and postoperatively
$C_2-(1-a)C_1$=facility of outflow of operated-on portion postoperatively
$aC_1/(1-X)$=facility of outflow of operated-on portion postoperatively

In setting up the second formula above the assumptions were made that resistance to flow through the meshwork and through the transcleral channels must be in series and that the circumferential flow in Schlemm's canal is negligible under the conditions of these experiments. Evidence to support the assumption of negligible circumferential flow is discussed in the text. Derivation was as follows:

$$\frac{aC_1}{(1-X)} = C_2-(1-a)C_1$$

$$X = 1-\frac{aC_1}{C_2-(1-a)C_1}$$

temperature of 24 C; this is equivalent to 0.19μl. at body temperature of 37 C after adjustment for the change in viscosity of water with change in temperature. To date a total of 52 enucleated normal human eyes have been measured by the perfusion method with no abnormal tension on the lens-iris diaphragm, and the average facility of outflow for all has been 0.176μl. at room temperature, or 0.23μl. at 37 C. The values in the present series therefore average a little lower than the majority, and it is

possible that eyes having mild open-angle glaucoma may have been included in this series, although there has been no basis other than low facility of outflow for suspecting it. In the Table Eyes 3 and 13, in particular, had unusually low facility of outflow.

In two eyes (Eyes 11 and 12) in which the trabecular meshwork was initially incised rather than excised (trabeculotomy instead of trabeculectomy) and perfusion was continued for an hour or more, the facility of outflow was found to be increased immediately following operation, but gradually with continuing perfusion the facility of outflow returned toward the preoperative values. In these eyes Schlemm's canal had been seen as a glistening white sulcus adjacent to the scleral spur when the trabecular meshwork was incised and the edges of the incision had been caused to gape by slight pressure of an instrument on the scleral spur or the adjacent ciliary body. However, after perfusion, during which the immediately increased facility of outflow gradually reverted toward the preoperative value, reexamination of the angle showed the edges of the incision to have come together again, either completely, so that the line of incision was scarcely discernible, or partially, so that the glistening white sulcus of Schlemm's canal was hidden irregularly by overlying fibers of trabecular meshwork. In these eyes the meshwork was thereafter actually removed so that it could not go back in place. Remeasurement showed again an immediate increase in facility of outflow, and under these circumstances it did not revert toward preoperative values during further perfusion.

An interesting phenomenon was noted on the surface of the globe when perfusion was carried out after the trabecular meshwork had been opened in only a fraction of the circumference. This consisted of a conspicuously augmented outflow of fluid in the vessels emerging from the sclera just in the region in which the meshwork had been operated upon. This localized increased outflow was made still more evident when fluorescein was added to the perfusing liquid. No outflow of fluorescein-tinted fluid was seen in the large emissary veins from the ciliary body, but the outflow was profuse from smaller vessels nearer the limbus, presumably aqueous veins and their anastomotic blood vessels. Not only was outflow in the region of opened trabecular meshwork greater than normal in volume, but in this region India ink was found to flow readily out of the anterior chamber and into the episcleral vessels.

Comment

The consistency with which the facility of outflow has been increased in the present series of experiments by trabeculotomy or trabeculectomy carried out by several different techniques, but all under direct microscopic control, indicated that the trabecular meshwork of enucleated normal eyes is in fact responsible for the principal resistance to aqueous outflow, as indicated in the foregoing section on results. Since in some of the experiments the instruments which were employed did not present a sharp or cutting edge to the scleral aspect of Schlemm's canal, it cannot well be supposed that collector channels were regularly cut into. Furthermore, in those experiments in which incision of the trabecular meshwork caused increase in facility of outflow only while the incision remained open, it appears that the status of the meshwork was directly related to the measured change in resistance. If incision into collector channels or a hypothetical secondary influence of tension in the trabecular meshwork upon the size of the collector channels were responsible for the observed change in resistance to outflow, these factors would not be expected to be reversible by reapposition of the cut trabecular meshwork fibers.

In retrospect it seems possible that in an earlier published series of experiments, in which the trabecular meshwork was curetted blindly with a needle, a failure to obtain an increase in facility of outflow in some instances may have been due to fail-

ure actually to obtain an effective opening of Schlemm's canal or due to reclosure of the opening by torn edges of the meshwork.[2] Microscopic examination of the eyes in those earlier experiments was carried out only after they had been fixed in formaldehyde and after most of the cornea had been excised by means of scissors. Possibly in this process the opening of Schlemm's canal came to appear more complete than it was at the time of operation and perfusion. The present series of experiments has been carried out with a much-improved technique, with microscopic examination at the time of measurement, and with much more consistent results.

If the present observations are applicable to open-angle glaucoma, it would appear that improvement of facility of aqueous outflow may be achieved surgically by making an opening into Schlemm's canal through the trabecular meshwork. In normal eyes it appears that opening the meshwork in only about one-sixth of the circumference can provide an increase of approximately 50% in facility of outflow. In eyes with open-angle glaucoma a similar or greater improvement may be expected if the abnormally poor facility of aqueous outflow which is characteristic of glaucoma is due to abnormality in the trabecular meshwork. Removal of meshwork would of course be less therapeutically effective if a greater proportion of the total resistance to outflow were found to be in the intrascleral channels in glaucomatous eyes. This remaining fundamental question can be resolved by determining the effect of trabeculotomy or trabeculectomy in enucleated eyes with open-angle glaucoma and by making comparison with the results obtained in the present experiments on normal eyes. Only the difficulty in obtaining eyes with well-authenticated open-angle glaucoma (uncomplicated by operation or other disease) delays this phase of the investigation.

Supposing, on the grounds mentioned in the foregoing paragraph, that the surgical procedure of opening the trabecular meshwork is desirable in the treatment of glaucoma, there are certain considerations in regard to the manner of accomplishment which the present experiments bring to light. It is, for instance, evident that to secure an effective and persistent opening from the anterior chamber to Schlemm's canal the incision must be accurately placed, and it must in some way be prevented from becoming obstructed by loosened strands of tissue which tend to be carried into the opening by the outflowing aqueous current. Not only is it desirable that the incision be confined accurately to the trabecular meshwork to avoid injury of the cornea anterior to Schwalbe's line and to avoid causing cyclogoniotomy posterior to the scleral spur but, further, it seems desirable that the opening be made in the portion of the meshwork which overlies Schlemm's canal. In the present study is has become apparent that incision just anterior to the scleral spur is regularly effective in opening into Schlemm's canal. The posterior portion of the canal as a rule lies just beyond the junction of scleral spur and trabecular meshwork, and it extends forward to approximately the midportion of the trabecular meshwork. In older persons there is commonly a narrow line of pigment in the trabecular meshwork at about its midportion or slightly posterior to this, suggesting that this is the portion of the meshwork through which most of the stream of aqueous humor normally flows, carrying with it occasional loose particles of pigment which become entrapped in the meshwork if they are too large to pass through. Microscopic examination of cross sections of the angle, as well as occasional gonioscopic views of blood refluxing into Schlemm's canal, show that this acquired pigment line (which is not to be confused with normally pigmented strands of uveal meshwork) lies directly in front of Schlemm's canal. From clinical study of patients by means of gonioscopy and tonography it has become evident that obstruction of this portion of the trabecular meshwork by peripheral anterior synechias may cause serious obstruction to outflow even when

the anterior quarter or third of the meshwork remains free. This clinical evidence suggests that the anterior edge of the meshwork does not provide ready access for aqueous humor to Schlemm's canal. Furthermore, incision of the meshwork near to Schwalbe's line in the present study did not facilitate aqueous outflow to the degree that incision of the posterior half of the meshwork did.

In clinical application of the information gained in the present experiments on enucleated eyes there are, of course, additional problems to be reckoned with in regard to the effect of healing of incised tissues and the possibility of blood refluxing into the anterior chamber from the opening in Schlemm's canal.

On the basis of information gained in the present study it is conceivable that good results which have been obtained clinically by goniotomy in congenital glaucoma may in some instances be attributable to incision of the corneoscleral trabecular meshwork itself, although the primary aim was to incise tissue overlying the meshwork. Consistent with this thought, I have seen buphthalmic eyes which have benefited from goniotomy and which appeared subsequently by gonioscopy to have an opening through the meshwork to the area where Schlemm's canal is normally located.

Academically, it is interesting that experimental opening into Schlemm's canal in only a fraction of the circumference does not increase facility of outflow as much as opening into the canal in the whole circumference. This indicates that the perfusion fluid in these eyes does not readily flow circumferentially in Schlemm's canal. In fact, both the approximate proportionality of fraction of circumference opened to degree of facilitation of outflow and the local character of augmented outflow observed on the surface of the globe indicate that the fluid encounters more resistance to flow along Schlemm's canal than to flow out through the transscleral vessels from Schlemm's canal in these eyes. Whether this differs from conditions in life is not known. The possibility exists that when the meshwork is opened the canal may inadvertently be occluded at the ends of the incision, but against this is the observation that outflow of fluorescein on the surface of the globe does appear to be augmented a short distance beyond the ends of the incision of the meshwork within the eye.

Summary

The influence of opening or removing the trabecular meshwork on the facility of outflow of intraocular fluid has been investigated in 16 enucleated normal human eyes, with use of more precise methods than have previously been used. By means of a new device which permits opening and tightly closing a hole in the cornea through which to dissect the trabecular meshwork under direct view with a microscope and to make measurements of facility of outflow by perfusion, it has been possible to open and remove the meshwork from within the anterior chamber and to determine that in every eye in this series this caused a significant increase in facility of outflow. It has been calculated that the trabecular meshwork as a whole in its relaxed state in these eyes was responsible for approximately 75% of the total resistance to outflow of fluid from the anterior chamber. It appears from the present series of experiments that trabeculotomy or trabeculectomy limited to a single quadrant may well be therapeutically helpful in open-angle glaucoma. However, a tendency for a cut in the meshwork to be partially self-sealing has been observed, and this is one of the factors to be considered and controlled in applying the results of this study to the surgical treatment of glaucoma.

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The Rule of Seven or Fourteen Thirds

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Often the refractionist finds the ophthalmometer value varying significantly from that of the proper correcting cylinder of the spectacle lens. Many still use the rule of Javal[1] to modify the ophthalmometer reading to approximate the proper cylindrical value of the correcting spectacle lens. In an article[2] published by the American Optical Company the rule of Javal is found faulty. The problem is analyzed, and, in a series of algebraic gymnastics, a formula is derived upon which are based the data in the Table, i. e., the upper figures in each row. These data represent corrected cylindrical components as derived from the influence of the spherical component upon the ophthalmometer reading and are for a vertex distance of 13.75 mm.

Neither the data of the Table nor the formula upon which they are based is easily committed to memory. The "Rule of 7 or 14 Thirds" is a useful approximation of these data, is much easier to remember, and can be used to "correct" ophthalmometer readings.

The "Rule of 7 or 14 Thirds" reminds one to multiply two fractions and then to algebraically add the product as a correction factor to the ophthalmometer reading. The first fraction has as its numerator the minus or plus spherical value; the denominator of this first fraction is either 7 or 14 (7 for minus spherical values and 14 for plus spherical values). The second fraction has as its numerator the number corresponding to the minus cylinder value obtained from the ophthalmometer; the denominator of this second fraction is 3 (the "thirds").

Although the reading of the previous paragraph may appear complex, a few examples will illustrate the simplicity and application of the "Rule of 7 or 14 Thirds."

EXAMPLE A.—An aphakic patient is found to have an ophthalmometer reading of -2.00×180. The spherical component is in the range of $+10$. What is the expected final cylinder in the spectacle lens?

$+10/14\times2/3=20/42$, or about $+0.47$

In the first fraction the numerator is $+10$ (the plus spherical value) and the denominator is 14 (14 for plus spherical values). In the second fraction the numerator is 2 (number corresponding to the minus cylinder value obtained from the ophthalmometer) and the denominator is 3 (the "thirds"). The correction factor of $+0.47$ is now algebraically added to the ophthalmometer reading of -2.00; thus, the expected final cylinder in the spectacle lens is -1.53 (compare this with the value in the Table of -1.52).

EXAMPLE B.—A 25-year-old patient with compound myopia astigmatism is found to have an ophthalmometer reading of -4.00×90. The spherical component is in the range of -6. What is the correction factor?

$-6/7\times4/3=-24/21$, or about -1.14

In the first fraction the numerator is -6 (the minus spherical value); the denominator is 7 (7 for minus spherical values). In the second fraction the numerator is 4 (number corresponding to the minus cylinder value obtained from the ophthalmometer); the denominator is 3 (the "thirds"). The correction factor of -1.14 is added algebraically to the ophthalmometer reading of -4.00 to give the

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Corrected Cylindrical Components as Derived from the Influence of the Ametropic Spherical Component upon the Ophthalmometer Reading *

Spherical Correction	Ophthalmometer Reading					
	−1.00 Ax	−2.00 Ax	−3.00 Ax	−4.00 Ax	−5.00 Ax	−6.00 Ax
	−1.25	−2.54	−3.87	−5.25	−6.67	−8.14
−8.00	−1.38	−2.76	−4.14	−5.52	−6.90	−8.28
	−1.22	−2.48	−3.78	−5.12	−6.50	−7.93
−7.00	−1.33	−2.66	−4.00	−5.33	−6.66	−8.00
	−1.19	−2.42	−3.68	−4.98	−6.33	−7.72
−6.00	−1.28	−2.57	−3.85	−5.14	−6.42	−7.71
	−1.16	−2.35	−3.58	−4.85	−6.16	−7.52
−5.00	−1.23	−2.47	−3.71	−4.95	−6.19	−7.42
	−1.13	−2.29	−3.49	−4.73	−6.00	−7.31
−4.00	−1.19	−2.38	−3.57	−4.76	−5.95	−7.14
	−1.10	−2.23	−3.40	−4.60	−5.84	−7.12
−3.00	−1.14	−2.28	−3.42	−4.57	−5.71	−6.85
	−1.07	−2.17	−3.31	−4.48	−5.68	−6.92
−2.00	−1.09	−2.19	−3.28	−4.38	−5.47	−6.57
	−1.04	−2.11	−3.22	−4.35	−5.52	−6.73
−1.00	−1.04	−2.09	−3.14	−4.19	−5.23	−6.28
	−1.01	−2.06	−3.13	−4.23	−5.37	−6.54
Zero						
	−0.99	−2.00	−3.04	−4.11	−5.22	−6.35
+1.00	−0.98	−1.96	−2.93	−3.91	−4.89	−5.86
	−0.96	−1.94	−2.96	−4.00	−5.07	−6.17
+2.00	−0.96	−1.91	−2.86	−3.81	−4.77	−5.72
	−0.93	−1.89	−2.87	−3.88	−4.92	−5.99
+3.00	−0.93	−1.86	−2.79	−3.72	−4.65	−5.58
	−0.90	−1.83	−2.79	−3.77	−4.78	−5.81
+4.00	−0.91	−1.81	−2.72	−3.62	−4.53	−5.43
	−0.88	−1.78	−2.71	−3.66	−4.63	−5.64
+5.00	−0.89	−1.77	−2.65	−3.53	−4.41	−5.29
	−0.85	−1.73	−2.62	−3.55	−4.49	−5.46
+6.00	−0.86	−1.72	−2.58	−3.43	−4.29	−5.15
	−0.83	−1.68	−2.55	−3.44	−4.36	−5.30
+7.00	−0.84	−1.67	−2.50	−3.34	−4.17	−5.00
	−0.80	−1.62	−2.47	−3.33	−4.22	−5.13
+8.00	−0.81	−1.62	−2.43	−3.24	−4.05	−4.86
	−0.78	−1.57	−2.39	−3.23	−4.09	−4.97
+9.00	−0.79	−1.58	−2.36	−3.15	−3.93	−4.72
	−0.75	−1.52	−2.31	−3.12	−3.95	−4.81
+10.00	−0.77	−1.53	−2.29	−3.05	−3.81	−4.58
	−0.73	−1.48	−2.24	−3.02	−3.83	−4.65
+11.00	−0.74	−1.48	−2.22	−2.96	−3.70	−4.43
	−0.70	−1.43	−2.17	−2.92	−3.70	−4.49
+12.00	−0.72	−1.43	−2.15	−2.86	−3.58	−4.29
	−0.68	−1.38	−2.09	−2.83	−3.57	−4.34
+13.00	−0.70	−1.30	−2.08	−2.77	−3.46	−4.15
	−0.66	−1.33	−2.02	−2.73	−3.45	−4.19
+14.00	−0.66	−1.33	−2.00	−2.67	−3.34	−4.00
	−0.64	−1.29	−1.95	−2.64	−3.33	−4.05
+15.00	−0.65	−1.29	−1.93	−2.58	−3.22	−3.86
	−0.61	−1.24	−1.89	−2.54	−3.21	−3.90
+16.00	−0.62	−1.24	−1.86	−2.48	−3.10	−3.72
	−0.59	−1.20	−1.82	−2.45	−3.10	−3.76
+17.00	−0.60	−1.20	−1.79	−2.38	−2.98	−3.58
	−0.57	−1.16	−1.75	−2.36	−2.99	−3.62
+18.00	−0.58	−1.15	−1.72	−2.29	−2.86	−3.43

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corrected value of −5.14 (compare this with the value in the Table of −4.98).

"Physiologic astigmatism" [2] is defined as the noncorneal astigmatism of the human eye probably caused by the crystalline lens. The allowance for such physiologic astigmatism is frequently noted as follows:

Age, Yr.	Cylinder		
Under 30	−0.25×90	or	+0.25×180
30 to 60	−0.50×90	or	+0.50×180
60 and over	−0.75×90	or	+0.75×180

Thus, in Example B the patient would have a final expected cylinder in the spectacle lens of −5.14×90 (corrected ophthalmometer reading by the rule of 7 or 14 thirds) plus −0.25×90 (physiologic astigmatism), or final expected cylinder of −5.39×90.

EXAMPLE C.—A patient between 30 and 60 years of age has an ophthalmometer reading of −3.00×180 and a spherical component in the range of +5. What is the corrected ophthalmometer reading and what is the final expected cylinder of his spectacles? By the "Rule of 7 or 14 Thirds" the correction factor is the following:

+5/14×3/3=15/42, or about +0.35

This correction factor added algebraically to the −3.00 of the ophthalmometer gives −2.65, the corrected ophthalmometer reading. The final expected correction cylinder would be −2.65×180 plus +0.50×180 (physiologic astigmatism), i. e., −2.15, or approximately −2.00 cyl, ax 180.

Comment

Review of the data in the Table reveals that for practical purposes and for the range of values covered in the Table the values obtained by the "Rule of 7 or 14 Thirds" for the most part approximate within 0.25 (although in some cases for 5 and 6 cylinders this is exceeded) those values obtained by lengthier computations (the lower figures in each row are values computed by the "Rule of 7 or 14 Thirds"). The rule does not apply when there is zero value for the spherical component.

There is some doubt that the variation of the physiological allowance is correlated with age exactly as is briefly tabulated. Still, the tabulations can be used as a guide.

Where there is an oblique astigmatism of the cornea, the application of physiological allowance as cited can be handled by known formulae treating combinations of obliquely crossed cylinders.

The values in the upper part of each row in the Table are for a vertex distance of 13.75 mm. The vertex distance in clinical practice will often vary somewhat from 13.75 mm. Notwithstanding, the data in the Table are useful, since they "correct" the omissions of Javal's rule and on theoretical basis are accurate. Since the use of these data requires reference to a chart or lengthier formula, these data are not widely used.

Conclusion

If one accepts the data in the Table (upper figures in each row), then the "Rule of 7 or 14 Thirds" for the range of values recorded is for clinical purposes also acceptable and equally applicable.

American Optical Company gave permission to use the data in Reference 2.

U. S. Naval Hospital, San Diego.

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Polyarteritis Nodosa with Ophthalmic and Neurological Complications

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The purpose of this paper is to report on a case of polyarteritis nodosa which has some unusual features of special ophthalmic and neurological interest.

Report of a Case

A woman was aged 35 years at death. At the age of 11 she had rheumatic fever; at 18 she had an appendectomy, and at 30, in her second pregnancy, she developed hypertensive toxemia necessitating termination during the 30th week. She had no other illnesses. Family history was not significant.

Since the age of 30 she had suffered from nocturnal cramps in the limbs of a myoclonic type and some loss of flesh on the left side of her face. At the age of 32 she developed abdominal pain in the epigastrium and vomiting of bilious fluid, which settled within a few weeks, and subsequently she had alternating periods of diarrhea and constipation. She lost weight. Soon afterward she was admitted to hospital for two weeks with a provisional diagnosis of mucous colitis. In general, the results of investigation were negative; the findings which have a bearing on the subsequent course were as follows.

Pulse and temperature were normal throughout the stay in hospital. Blood pressure after rest was 160/90. There was abdominal tenderness in the right hypochondrium. The central nervous system was normal. X-rays of chest, of stomach and duodenum, and of gallbladder were normal. Barium enema was reported as showing slight hesitation at both the splenic and hepatic flexures and considerable loss of haustration in the descending and sigmoid colon. In films after evacuation an abnormal mucosal pattern was noted throughout the entire colon, which was considered compatible with mucous colitis. Catheter specimen of urine showed a trace of albumin, a few pus and epithelial cells, and no growth on culture. Blood cell count showed Hb. 96% and total leukocytes 8800 per cubic millimeter, of which 70% were neutrophils, 2%, eosinophils; 6%, large mononuclear cells, and 22%, lymphocytes. Examination of feces for occult blood was negative.

At the age of 33 there was a recurrence of diarrhea, vomiting, and loss of weight, and the patient was readmitted to hospital. Again, although she looked ill, no abnormal physical signs were detected, and in a few days the abdominal symptoms settled. She was again afebrile, and investigations showed the same findings as above. However, the erythrocyte sedimentation rate (ESR) was 40 mm. in an hour. A month later, eye symptoms commenced, first as teichopsia and within three weeks as defective vision in the right eye. Visual acuity was then 6/36 in the right eye and 6/5 in the left eye. The fundus of the left eye was normal. In the right eye there was a small area of perivasculitis of the retina around the macula and, near it, there were two or three small white exudates no larger than miliary tubercles of choroid. There

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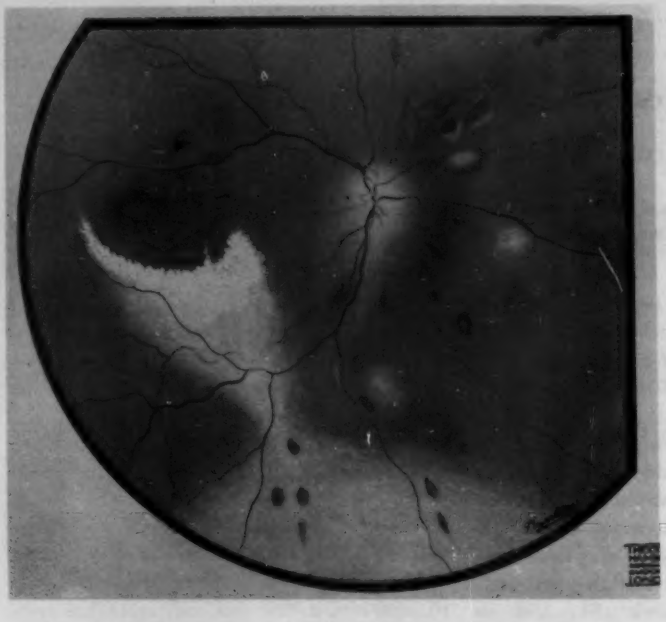

Fig. 1.—Fundus of right eye.

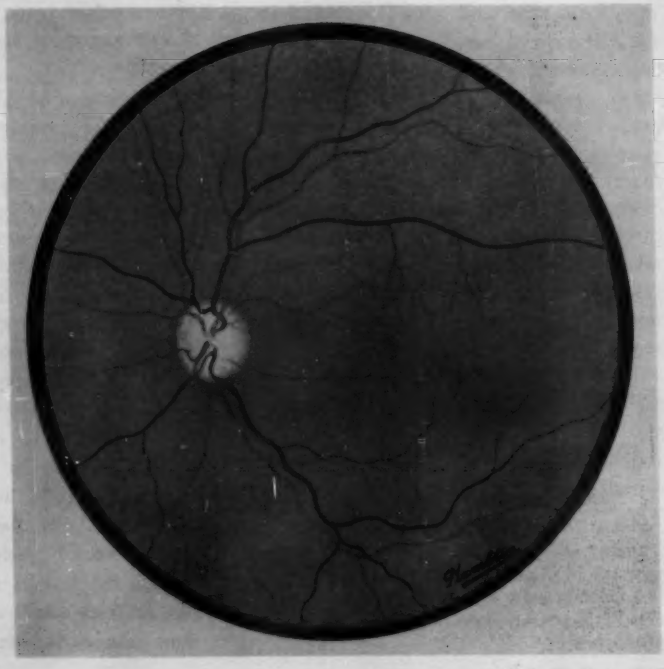

Fig. 2.—Fundus of left eye.

was at that time no edema of the retina or of the optic disc. The patient was kept under observation for five months, during which time the acuity of the right eye gradually became less than the ability to count fingers. Keratic precipitates (K. P.) developed in it, remaining constantly thereafter, and also striking changes in the fundus. These consisted of multiple retinal hemorrhages, massed white exudates around the macula, retinal edema and papilledema, and a considerable amount of raised white exudate inferiorly projecting 10 D. (Fig. 1). By contrast, the appearances of the left eye, both by ophthalmoscope and by slit lamp, remained entirely normal throughout the illness (Fig. 2). Further investigations were done during this period. Several examinations of the urine were normal, and the fasting blood sugar was 88 mg. per 100 ml. The Wassermann and Kahn reactions were negative. Intradermal and agglutination tests for brucellosis and Mantoux reaction (1:1000) were negative. Plain radiographs of the chest and of the hands and feet were normal. A biopsy of gastrocnemius muscle was reported as negative for any sign of polyarteritis. No subcutaneous nodules were found. The total serum protein was 6.0 gm/100 ml., of which albumin was 3.4 gm.; globulin, 2.6 gm. ESR was examined at intervals of 10 weeks and gave successive readings of 30, 49, and 45 mm. in an hour.

At the age of 34 she had noticed some weakness and cramp of the left leg, and she was readmitted to hospital. On examination, temperature was 98 F; pulse rate, 72, and respirations, 19 per minute. She was a frail, thin woman, weighing 105 lb., with an icteric appearance, though without jaundice. There was undue prominence of the bony architecture of the left side of the face and head, owing to the absence of subcutaneous fat. Vision of right eye was counting fingers; that of the left eye, 6/6 and J 1, and the eye changes were as described above. There were hypoalgesia and hypoesthesia of the left trigeminus nerve, second and third divisions; the remainder of the cranial nerves and the upper limbs and right lower limb were normal. There was some wasting of the left quadriceps and calf muscles and moderate weakness of the left lower limb, especially of the hip flexors, knee extensors, and ankle dorsiflexors. The left knee and ankle jerks were absent. The abdominal reflexes were preserved, and the plantar responses were flexor. There were hypoalgesia and hypoesthesia of the dermatomes L 1 to L 5, inclusive, on the left side. No other abnormal physical signs were detected; serial blood pressure readings ranged from 120/70 to 110/60 mm. Hg. The ESR was 35 to 55 mm. in an hour throughout her stay in hospital. The cerebrospinal fluid pressure and manometric observations were normal, and the fluid contained no cells and 28 mg. of protein per 100 ml.; Lange test showed no change; the Wassermann reaction was negative in both blood and cerebrospinal fluid. The Mantoux reaction was negative at 1:100, but the Kveim intradermal test was negative on two occasions. Additional investigations included full blood cell counts; blood cultures; chemical and cytological examination of urine; tests of glucose tolerance, renal and hepatic function, and fat balance; plain radiographs of chest, cranium, abdomen, and hands, and a barium meal. All these were normal.

In view of the likelihood of a collagen disease the patient was given cortisone, 37 mg. daily for 17 days, but without obvious benefit, and was discharged from hospital. As regards the eye, after a short quiet period, the changes in the right eye became, if anything, more acute. A flare developed in the aqueous fluid for two weeks, fresh keratic precipitates were deposited, and small vitreous hemorrhages occurred. The vitreous then cleared, revealing the gross fundus changes still spreading. On two occasions she complained of intense pain in the eye during the night, and, in view of the possibility of a secondary glaucoma, the

eye was enucleated. At this time the ESR was 42 mm. in an hour.

Three months later (aged 35) she complained of lassitude, loss of weight, and swelling of the legs, and vomiting started again. She was readmitted to hospital. She now had intermittent pyrexia (temperature of 102 F), which continued till the time of her death, three months later. The pulse rate was 120 on several occasions, and respirations averaged 28 per minute. Both legs were edematous. Neurological examination showed no extra physical signs. Respiratory and cardiovascular systems were normal. The abdomen was distended with gas and showed visible peristalsis.

Results of investigations were as follows: Blood cell count: Hb., 67%; RBC, 3,400,000 per cubic millimeter; color index, 0.9; WBC, 14,000 per cubic millimeter, of which 85% were neutrophils; 3%, eosinophils; 9%, lymphocytes, and 4% monocytes. ESR was 45 mm. in an hour. The plasma proteins were 5.7 gm/100 ml., of which albumin was 2.3 gm. and globulin, 3.4 gm. Plain radiographs of chest, the blood electrolytes, and examination of urine and feces were normal. Sigmoidoscopy revealed a reddened and granular mucosa, but without ulceration.

She was treated empirically with chlortetracycline (Aureomycin), 1000 mg. daily, which five days later was replaced by penicillin, 500,000 units, and streptomycin 1 gm. daily, but without any reduction of pyrexia. Constipation and profuse vomiting then occurred, and a laparotomy was done. The peritoneal cavity contained some straw-colored fluid. The jejunum was grossly dilated. The whole of the ileum, except the terminal 4 in., was abnormal; its wall was irregularly thickened and constricted, like a string of sausages, but collapsed and purplish. The cecum, the ascending and descending part of the colon, was thin-walled and dilated, while the descending colon and rectum were empty, though there was no abrupt constriction of the splenic flexure. Liver, spleen, and other abdominal organs appeared normal. An enterocolostomy was performed and a small piece of small intestine, as well as a portion of rectus abdominis muscle, was sent for histological examination. The muscle was reported to be normal; the intestinal biopsy specimen showed an increased number of eosinophils in the perivascular tissue of all layers.

The postoperative course was complicated by a transient fecal fistula. On return to the medical ward the patient was found to have signs of peripheral neuropathy, i. e., hypotonia, wasting and weakness of peripheral limb musculature, areflexia and glove-and-stocking cutaneous sensory loss. She was treated with cortisone, 300 mg. daily, and corticotropin (ACTH), 25 mg. by injection twice weekly. This had no beneficial effect, and she went into status epilepticus. This was controlled first by soluble phenobarbital and then by the same drug orally and diphenylhydantoin (Phenytoin) sodium. From this time on, she became drowsy and delusions developed. An electroencephalogram showed evidence of a discharging focus in the left temporal lobe. Status epilepticus returned, and the patient became disorientated, lapsed into stupor and coma, and died.

Necropsy.—The body was that of an emaciated young woman. The right eye had been enucleated. There was an ill-defined parchment-like area of skin, 2.5 cm. diameter, in the central part of the brow.

Internal examination revealed noteworthy changes only in the organs detailed below. There was no sign of rheumatic endocarditis or of pulmonary tuberculosis. Loops of ileum were adherent to the anterior abdominal wall in the region of the surgical incision and to the ascending colon. The mucosal aspect of the ileum showed numerous transverse bands of thickening, as many as five in 1 ft., each about 3 mm. in thickness. They appeared to be scars and caused a moderate narrowing of the lumen. The intervening mucosa had an

abnormal wrinkled appearance. At the site of adhesion to the ascending colon the ileum had three ulcers, with serpigenous outline and slightly raised margins. No tubercles could be recognized here or elsewhere. The mesentery contained one calcified lymph node. Other lymph nodes were prominent but showed no evidence of tuberculosis. The kidneys showed very sparse ischemic scars in the cortices. The bone marrow was red in the distal half of the femur. The lungs showed basal edema and bronchopneumonia.

The brain was examined after fixation. The basal meninges and those overlying sulci on the vertex showed slight fibrous thickening. The cerebral gyri comprising the inferior parietal lobule on the right side were swollen, soft, and discolored brown. On coronal section, this lesion overlay an old hemorrhage 4 cm. in diameter, which involved gray and white matter and had a reddish-brown capsule 1 mm. thick. Cortical softening affected also both temporal lobes and zones up to 3 mm. in diameter in the inferior frontal and lateral occipital regions. The vessels comprising the circle of Willis and its main branches were carefully scrutinized, without revealing even slight mural thickening or thrombosis. The spinal cord appeared normal.

Histology.—Brain: The "hemorrhage" in the right parietal region consisted centrally of necrotic cerebral tissue, including compound granular corpuscles, mixed with masses of depigmented red cells. It was therefore hemorrhage into an area of softening. The remaining softenings were of varied appearance. Some of the large areas of infarction, for example, at the tip of the right temporal lobe, showed a typical condensation in thickness of the gray matter, with loss of the normal laminar pattern. There was an outer molecular and inner zone of proliferating swollen (gemästete) astrocytes, enclosing a band of proliferating capillaries with swollen endothelium. There was no cavitation. The underlying white matter showed reactive gliosis. Another large area of infarction affecting the insular cortex showed loss of nerve cells only, with masses of reacting swollen astrocytes in all laminae. Many of the infarcts were of microscopical dimensions. In the putamen they either consisted of masses of compound granular corpuscles or were older and showed cavitation. In the frontal cortex, on either side of the median fissure, there were wedge-shaped areas, based on the surface, where nerve cells had disappeared and there was reactive gliosis.

The blood vessels were of peculiar interest. Random sections through the large arteries at the base of the brain and in the cerebral sulci showed entirely normal walls, with a single internal elastic lamina. Many small arteries, however, of outside diameter 100μ to 200μ, showed a considerable alteration of their wall. The elastic and muscle coats were lost and were replaced by concentric rings of anuclear thick collagen. In most instances the collagenous fibers were separated, leaving a wide space between the innermost and outermost fibers. The endothelium represented by one or two pyknotic nuclei, enclosed a lumen reduced to a diameter often a quarter of that of the vessel as a whole (Fig. 3). The affected arteries were not ringed by lymphocytes unless they were in a territory of drainage adjoining an infarct, in which case the perivascular space contained a small number of these cells. In no instances were inflammatory cells found within the wall. The wall thickness and the situation of the vessels suggested that they were arteries, although on occasions it was not possible to exclude a venous nature. The small arteries affected in this way were chiefly, but by no means exclusively, in the region of the infarcts and dipping into the infarcted cortex. In the putamen arterioles often had thickened, partly hyaline walls. In the cortex some precapillaries showed fibrosis.

Spinal Cord: The meninges in sections taken at representative levels showed slight fibrous thickening. Occasional small arter-

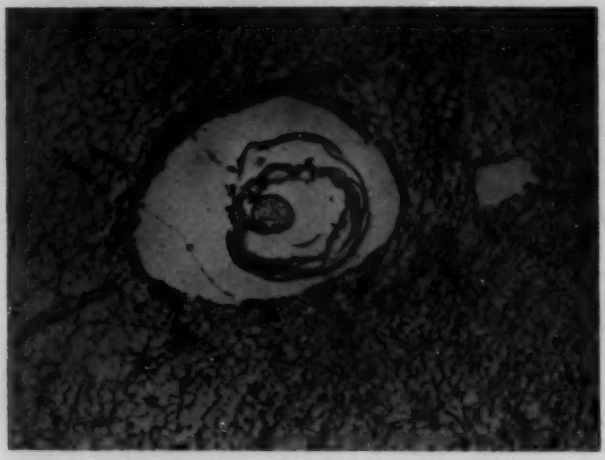

Fig. 3.—A small perforating cerebral artery with an area of tissue rarefaction. The wall is composed entirely of collagenous fibers, and the lumen is much reduced. Hematoxylin and eosin; × 155.

ies had slightly thickened collagenized walls, infrequently surrounded by a sparse lymphocytic infiltrate. Dorsal nerve roots occasionally showed patches of atrophy, surrounding small collagenized arteries. The cord itself appeared normal.

Peripheral Nerve (Sacral): No changes.

Muscle (Left Leg): There was obvious neurogenic atrophy, with reduction in size of many fibers, approximation of subsarcolemmal nuclei, and occasionally nuclear clumping. There was no replacement fibrosis. Groups of normal fibers with preserved striations stood out in contrast to the atrophic fibers. Many small arteries had somewhat thickened walls in which the muscle coat remained prominent. One small artery showed subintimal and periadventitial fibrosis, with a perivascular infiltration of lymphocytes (Fig. 4).

Skin: There was some flattening of the epithelial papillae and prominence but not degeneration of the collagen in the corium. The vessels were normal. The appearances were consistent with a mild degree of scleroderma.

Ileum: There were widespread areas of fibrosis, in which the muscle coat and mucosa were destroyed and fibrous tissue spread out beyond the confines of the serosa.

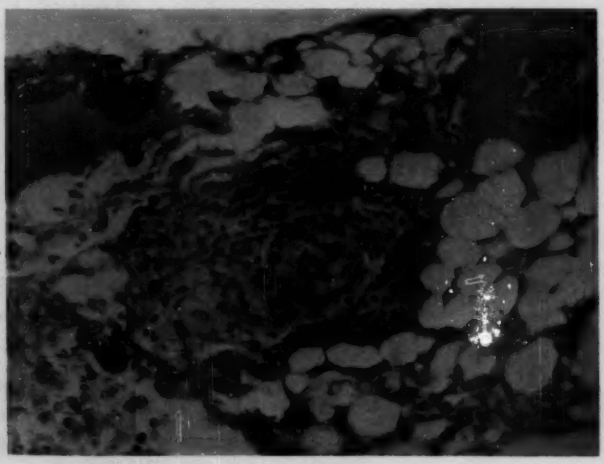

Fig. 4.—Small artery in muscle showing adventitial lymphocytic infiltration and intimal thickening. Hematoxylin and eosin; × 250.

The fibrosis was nonspecific, and there were no lesions to suggest tuberculosis. The small vessels did not show any change.

Kidney, Spleen, and Pancreas: These organs were normal, and their blood vessels were normal.

Histopathology of the Eye.—The right eye was sent to Dr. Norman Ashton, at the Institute of Ophthalmology, London, who reported as follows:

"*Horizontal Sections.* The cornea shows peripheral inflammatory infiltration, and there are a few keratic precipitates present. The whole of the anterior uvea is mildly infiltrated with chronic inflammatory cells of which plasma cells predominate. The uveal meshwork contains aggregations of inflammatory cells, but the filtration angles themselves are patent. Posterior synechiae are present. The lens is normal. The main pathological changes are in the posterior half of the eye where the choroid shows discrete focal aggregations of pale endothelioid cells surrounded with lymphocytes, plasma cells and eosinophils; a similar focus is seen at the root of the iris on one side [Fig. 5]. These infiltrations show no particular relationship to vessels and closely resemble those seen in sarcoidosis. In several areas this inflammatory tissue has eroded through Bruch's membrane to form small pigmented nodules similar to those of Dalen-Fuchs.

"The retina is shallowly but extensively detached and shows marked perivasculitis, oedema, intraretinal haemorrhage and numerous exudates. The most striking changes in the retina, however, are to be seen in the retinal vessels, which show the various stages of polyarteritis nodosa or necrotizing arteritis [Fig. 6]: in some the walls are uniformly thickened with subendothelial fibrin, in others fibrin has exuded through the vessel walls to form extramural foci, while the most severely affected vessels show necrosis, considerable disorganization and recanalization. All the vessels are surrounded with an intense inflammatory infiltration in which eosinophils predominate. These vascular changes are active, acute and clearly of a specific character and are not secondary to a granulom-

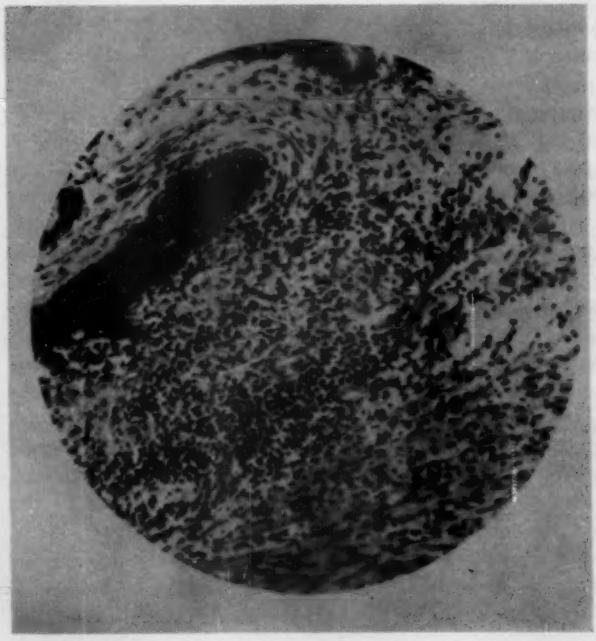

Fig. 5.—Granulomatous focus at the root of the iris. Hematoxylin and eosin; × 125. (Photomicrograph by Leeds University photography department.)

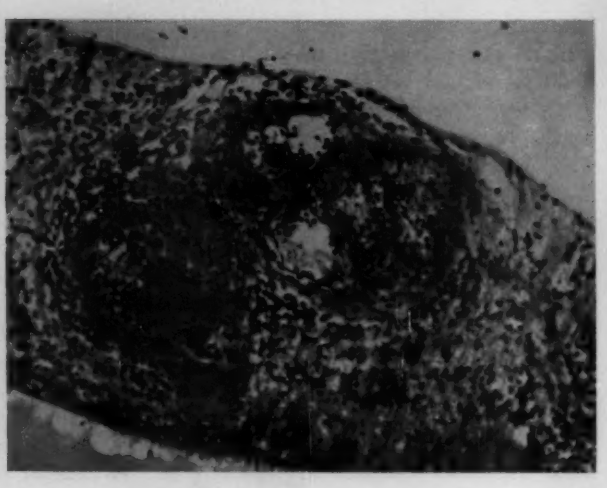

Fig. 6.—Retina showing polyarteritis or necrotizing angiitis. Hematoxylin and eosin; × 160.

atous choroiditis. No toxoplasma or other organisms could be found in specially stained sections.

"Polyarteritis nodosa of retinal vessels. Granulomatous uveitis."

Comment

Until the eye sections were available, the pathological diagnosis remained in doubt. The skin changes, the widespread fibrosis in the ileum, the multiple cerebral infarcts, and the neurogenic muscular atrophy incriminated some form of disseminated vascular disease. The ileal fibrosis was peculiar, but fibrotic lesions of the alimentary tract develop in 50% of cases of polyarteritis nodosa (Miller and Daley, 1946), and superficial erosion and ulceration may occur.

The neurogenic muscular atrophy was presumably due to involvement of peripheral nerves by vascular disease, although this was not proven by examination of the nerve segments available. Apart from the eye, however, there were no acute specific changes in the arteries in any of the organs examined. The retinal and choroidal lesions indicated without doubt a necrotizing polyarteritis and gave a clue to the disease process affecting the patient as a whole. The present case is unusual in the restriction of the acute vascular lesions to the eye. This raises the problem of whether the scarred, collagenized small cerebral arteries may have been related to previous attacks of polyarteritis. No acute vascular lesions were found in the brain, in spite of thorough search. Scarred arteries of this sort can sometimes be seen in the neighborhood of cerebral infarcts due to thrombosis of large arteries in older patients. In the present case, however, the changes are particularly marked and had occurred in a young subject without arteriosclerotic changes in the larger arteries. They were by no means confined to the infarcts. It is reasonable to assume that the infarcts, large and small, were the result of the sclerosis of these numerous small arteries.

Miller (1949) concluded that polyarteritis nodosa was an allergic response of anaphylactoid type to a variety of antigenic agents, usually, but not invariably, bacterial. The onset of the disease often follows an infection or drug intoxication; many of the affected subjects give a history of other conditions, such as acute rheumatism or nephritis, which are probably determined by hypersensitivity; allergic phenomena, such as asthma or urticaria, frequently occur during the course of the malady. Rich (1942) and Gregory (1943) provoked an acute necrotizing arteritis in rabbits, which closely resembles polyarteritis in man, by the repeated injection of foreign

protein. Gillman and Gilbert (1956) produced a necrotizing angiitis like polyarteritis in rats by an intensive vitamin D diet. Our patient gave a history of rheumatic fever and of toxemia in pregnancy, but no other allergic, toxic, or infective factors were identified.

Miller and Daley (1946) reviewed the clinical aspects of polyarteritis nodosa as recorded in the world literature. The commonest features included fever with disproportionate tachycardia, vascular hypertension, polymorphonuclear leukocytosis, elevation of the ESR, albumin and blood in the urine, and edema. Our patient showed only one of these signs, a raised ESR, in the early stages, and so the diagnosis was initially difficult. Later she developed pyrexia, leukocytosis, edema and also reversal of the albumin-globulin ratio, hypochromic anemia, and intestinal and central nervous complications. Furthermore, lipodystrophy of the face had not hitherto been recorded as occurring in association with polyarteritis nodosa, and its presence was considered to favor a diagnosis of diffuse scleroderma or systemic sclerosis, particularly in view of the coexistence of intestinal lesions. Abdominal changes do, however, occur in a large proportion of polyarteritis cases. Sorsby (1951) puts it as high as 60%; Kernohan and Woltman (1938) and Miller and Daley (1946), as about 50%; Davson, Ball, and Platt (1948) say that "very frequently there is abdominal pain, sometimes with symptoms of perforation, peritonitis, or mesenteric thrombosis," and in five out of eight of their fully recorded cases abdominal symptoms presented. Goldstein and Wexler's (1929) case had marked abdominal signs.

Ophthalmic involvement is present in 10% to 20% of polyarteritis cases. Usually it is an indirect effect and is due to the frequent renal involvement in the disease; Sampson (1945) and Wise (1952) point out that there are still very few published cases in which ophthalmoscopy has shown more than an ordinary albuminuric retinopathy. The direct involvement of uveal or retinal arteries by the inflammatory lesions of polyarteritis has not been recorded very often. There was an isolated brief description of it in the 19th century by Müller (1889), though without illustrations; his paragraph describing it is quoted by Goldstein and Wexler (1929), from which it appears definite that the retinal arteries were directly affected by the inflammatory process. Thereafter little was heard of it until Hassen and Knack (1917) reported several cases of nephritis with or without albuminuric retinopathy in which they found unexplained inflammatory lesions in the choroidal vessels of the eye. Friedenwald and Rones (1931) briefly mention this report and raise the question whether cases of polyarteritis might have been included in Hassen and Knack's series.

Christeller (1926) described inflammatory changes in the choroid in polyarteritis. Böck (1929) and von Herrenschwand (1929) independently illustrated lesions of polyarteritis in the ciliary arteries, but not intraocularly. Then Goldstein and Wexler (1929) reported the first complete study with photomicrographs of intraocular lesions in polyarteritis, and this work was followed by that of Friedenwald and Rones (1931) and Helpern and Trubek (1933). It is of interest that in none of these three reports did a search reveal any trace of inflammatory involvement of the retinal arteries, only of the choroidal vessels. This frequent involvement of the choroid is mentioned in recent reviews by Kernohan and Woltman (1938), Wise (1952), and Herson and Sampson (1949), and the last-mentioned authors consider that it may be due to the predilection of polyarteritis for vessels of medium size, there being a greater number of such vessels in the choroid than in the retina. Exudative retinal detachments, which may vary from time to time, have been recorded by Kernohan and Woltman (1938) (Case 5), by Sampson (1945), by Herson and Sampson (1949), and by Davson, Ball, and Platt (1948) (Case 13). Our

case also showed keratic precipitates and synechiae; the occurrence in polyarteritis of this anterior uveitis is reported by several authors, e. g., King (1935); Sampson (1945); Miller and Daley (1946); Davson, Ball, and Platt (1948) (Case 8), and Goar and Smith (1952).

The formation of granulomatous foci in polyarteritis was shown by Pagel (1951) to occur in relation to blood vessels. As regards the eye, Helpern and Trubek (1933), after describing lesions of polyarteritis in the choroidal arteries, go on to say that "adjacent to and apparently associated with some of the necrotic arteries, small granulomatous-like nodules were found composed of fibroblasts and endothelial cells with a scant infiltration by polymorphonuclear leucocytes and capillary formation." Their Figure 10 gives an excellent picture of a granulomatous nodule in the choroid. More recently Boeck (1956) described granulomas developing in relation to affected choroidal vessels. In our case, Dr. Ashton has pointed out that they also occurred chiefly in the choroid and in the root of the iris.

The neurological complications of polyarteritis nodosa have been reviewed by Kernohan and Woltman (1938), Foster and Malamud (1941), and Miller and Daley (1946). Thrombosis of vessels in the cerebrum, brain stem, or spinal cord may give rise to focal neurological signs, and, less often, hemorrhage may arise from an aneurysm into the cerebral substance or subarachnoid space. Fits occur in 15% (Harris, Lynch, and O'Hare, 1939), while dementia, confusional psychosis, and coma, unrelated to uremia, are fairly common. About 20% have peripheral neuropathy (Kernohan and Woltman, 1938; Jones, 1940; Miller, 1949), either mononeuritis multiplex or polyneuritis. Though such may be due to toxemia, Miller (1949) has shown that the vasa nervorum may be involved by obliterative arterial disease. Our patient had mononeuritis multiplex with involvement of the trigeminus and the lumbar nerve roots and later developed polyneuritis, epilepsy, and confusional psychopathy and died in coma.

Summary

A case is reported of a woman 35 years of age who for four years previously had progressive loss of subcutaneous tissue of the left side of the face and for 3½ years previously had intermittent diarrhea, vomiting, and loss of weight.

During the three years she was under observation she developed symptoms and signs referable to one eye consisting of loss of vision, retinal perivasculitis, and iridocyclitis going on to gross retinal exudates and hemorrhages and papilledema. She also developed various neurological syndromes, including sensory neuropathy of the trigeminus, lumbar radiculopathy, polyneuritis, epilepsy, and a confusional psychopathy terminating in coma. Furthermore, there developed intestinal obstruction due to a diffuse pathology of the ileum and colon. Extensive investigations were done, and they were for the most part negative, though the ESR was persistently raised. During the terminal stages of her illness she had intermittent fever, leukocytosis, and inversion of the albumin-globulin ratio and failed to improve when she was given antibiotics or cortisone. The general postmortem findings indicated some indeterminate form of disseminated vascular disease, but the histopathology of the involved eye revealed that the basic condition was, in fact, active polyarteritis.

Dr. Hugh Garland gave advice in the preparation of this paper, access to the patient, who was admitted under his care; Messrs. Hamblins, London, provided the fundus paintings.

39 Micklegate.

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Recovery of Toxoplasma from a Human Eye

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Following the discovery of human congenital toxoplasmosis, in 1937, it was suspected by ophthalmologists and other investigators that Toxoplasma might be a cause of acquired chorioretinitis. It was not until 1954 that Jacobs [1] reported the isolation of these organisms from the enucleated eye of an adult. The strain isolated from his patient was of high virulence for laboratory animals, closely resembling the well-known RH strain.

In this paper we report the characteristics of a strain exhibiting low virulence for animals which was recovered from the enucleated eye of a 20-year-old youth. A diagnosis of congenital ocular toxoplasmosis had been made on this patient eight years previously.

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From the Francis I. Proctor Foundation for Research in Ophthalmology, University of California School of Medicine.

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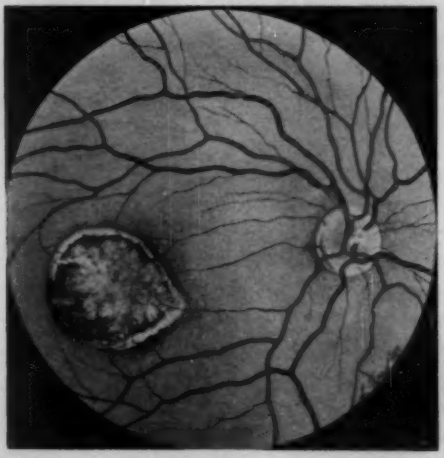
Fig. 1.—Healed macular chorioretinitis, right eye, due to congenital toxoplasmic infection.



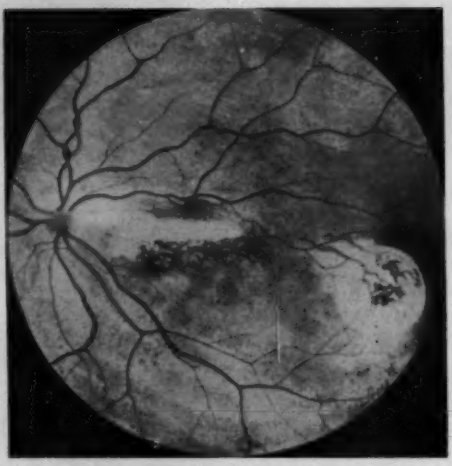


Fig. 2.—Acute chorioretinitis, left eye. Note old healed lesion in fundus periphery.

Report of a Case

A white boy aged 12 years was first seen in March, 1948, because he had sudden diminution of vision in his good left eye. During a routine clinic examination at the age of 1½ years he had been found to have a chorioretinal scar in the macula of the right eye. There were no further eye difficulties until the present disturbance in the left eye.

Upon examination, the right vision was 15/200 and the left was 20/200. The right macula (Fig. 1) had a large area of healed chorioretinitis. The left eye (Fig. 2) showed a heavy vitreous haze and an indistinct massive yellowish area of inflammation extending from the disc to the temporal periphery. X-rays of the skull showed calcifications (Figs. 3 and 4). The neutralizing antibody test and the skin test were

Fig. 3.—Lateral skull x-ray, showing calcifications.

positive. General physical examination was negative. There was no mental retardation or other effects of the congenital infection.

Course.—Five months after the onset the left vitreous had cleared so that vision was 20/70. Two and one-half years later the lesion was fairly well healed, leaving extensive scarring and pigmentation of the entire temporal fundus. The vision was 20/100. The methylene blue dye test was positive at a titer of 1:128.

For several years, relapses occurred in this eye, so that the vision was lost. The eye became divergent and hypotonic, developed a cataract, and commenced to shrink. The patient requested enucleation, which was done in June, 1956. The dye-test titer one year after enucleation was 1:16, and the skin test was still positive.

Gross Examination.—The opened eye revealed a large yellow-white thickened area of chorioretinitis in the temporal portion extending from the optic disc almost to the ora serrata (Fig. 5). It was hemorrhagic in areas and was elevated by organizing exudate (Fig. 6). There was minimal disturbance of the pigment. Approximately three-fourths of the eye was used for cultures, and the other quarter, for histological study.

Microscopic Examination.—The sections include a portion of the infected tissues from the temporal side. Included in the specimen are some sclera, choroid, and retina. One section is composed only of choroid and retina, which show marked scarring and early bony degeneration (Fig. 7). Some portions of the retina still show rather extensive necrosis with secondary gliosis. The acute phase of the inflammation, however, appears to have healed by this time.

Another specimen shows a portion of retina and choroid which are firmly bound to each other by a dense scar (Fig. 8). The retina is thickened and shows extensive gliosis. The vessels are congested, and there is migration of pigment. Some of the subretinal scar tissue shows numerous areas of calcareous and bony degeneration.

The third specimen is composed of choroid, retina, and a portion of sclera. There is extensive thickening, disorganization, and scarring of the retina. Some of the

Fig. 4.—Anteroposterior skull x-ray, showing calcifications.

Fig. 5.—Anterior half of the eye, showing the lens. Note the peripheral chorioretinal scarring near the edge of the tissue.

vessels are obliterated as a result of the intense inflammation. There is a healed chorioretinal scar without residual inflammation.

Serial sections of these specimens failed to show Toxoplasma, possibly because such a small specimen was used for microscopic studies.

Cultural Studies.—Mice: The chorioretinal tissue from the back of the eye was ground in a mortar, diluted with saline, and inoculated intraperitoneally into four mice. Two of these mice were also given inoculations intracerebrally with 0.03 ml. of the suspension.

The mice did not show signs of illness. In three weeks their brains were removed, ground, and subinoculated intraperitoneally into four other mice (Subinoculation 1). Six days later these mice showed some signs of illness, and it was possible to aspirate a small amount of peritoneal fluid. A few organisms were found which strongly resembled Toxoplasma. Other mice were given inoculations of this fluid (Subinoculation 2).

Fig. 6.—Posterior half of eye, showing retinal thickening, elevation, and fibrosis, with organizing subretinal exudate. Note disc, with vessels radiating to the quadrants.

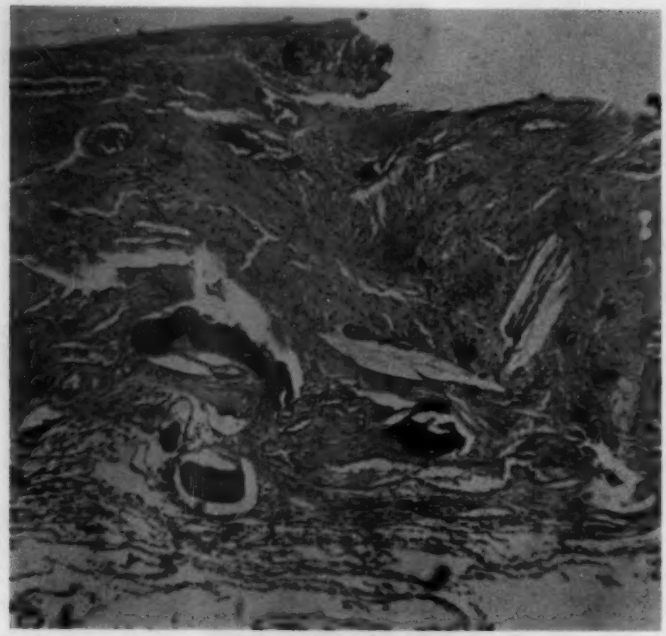


Fig. 7.—Chorioretinal scarring in the posterior portion of the eye. Note calcareous degeneration.

Fig. 8.—Chorioretinal scarring in a portion of the posterior part of the eye.

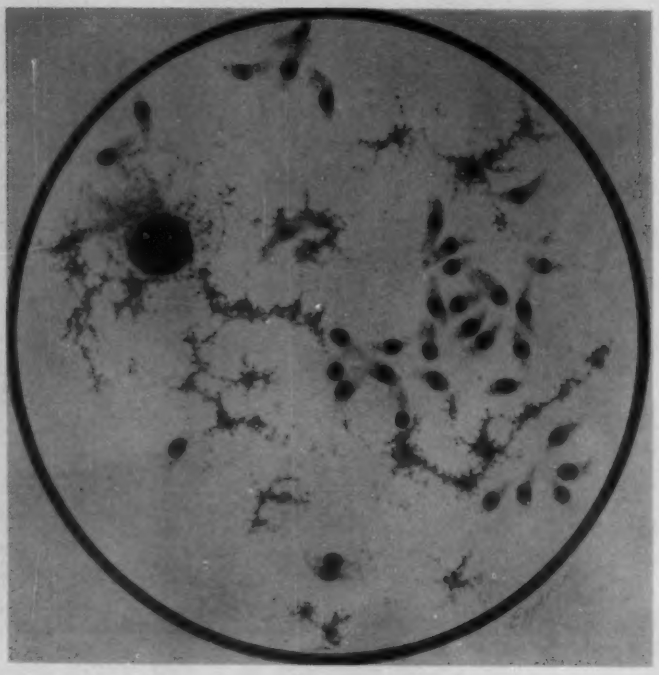


Fig. 9.—Mouse peritoneal fluid three days after inoculation, showing extracellular Toxoplasma organisms.

Eight days later these mice became somewhat ill, and organisms resembling Toxoplasma could be demonstrated in the peritoneal fluid (Fig. 9). Several new mice were given inoculations of this material and within a week became ill. All recovered (Subinoculation 3).

Seven months later the brain from one of these mice was inoculated into other mice (Subinoculation 4). Twelve days later they were very sick. There were not enough Toxoplasma organisms in the peritoneal fluid to run the dye test, but transfers were continued. When the mice were killed, 10 months later, the pooled sera from these mice showed a dye test titer of 1:1024.

The 11th subinoculation produced sufficient peritoneal fluid in four days to use as antigen in the dye test. However, there were too many unstained (probably dead) organisms present, which made the negative control too high for a satisfactory test.

The mice of Subinoculations 12 and 13 were sick in three days, and abundant peritoneal fluid was obtained. In tests with these exudates the negative controls were satisfactory but the positive ones were not. When we used the RH strain as antigen for comparison, the titers with the RH strain were at least fourfold higher than with our strain (designated MF). Otherwise, the staining characteristics were the same as those of the RH strain.

After additional subcultures (18 months after enucleation) the strain has increased in virulence to the point where mice become nearly as ill in three days as those given inoculations of RH strain (Fig. 10). The peritoneal fluid, however, is still not satisfactory for the dye test and differs from that of the RH strain in being more mucoid and containing more clumped cells and debris. It also differs from that produced by another strain of low virulence previously recovered from a spontaneously infected guinea pig. (This latter strain causes a large amount of rather watery peritoneal fluid with few organisms and many cells, whereas both the RH and the

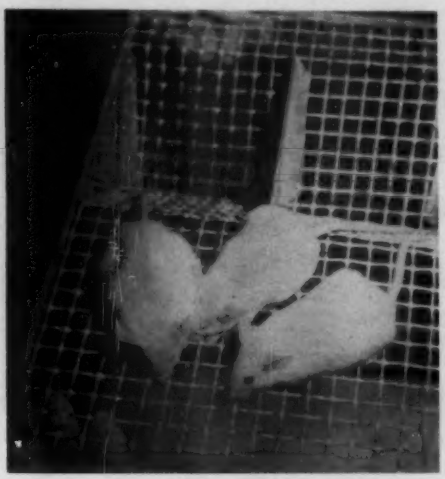


Fig. 10.—One of the mice on the left has been given an inoculation of the RH strain, and one, of the MF strain. They appear equally sick three days after inoculation. The mouse on the right has not been given any inoculation.

MF strain show many organisms and fewer cells.)

Four mice given inoculations on Sept. 20, 1957, of peritoneal fluid of Subinoculation 7 recovered and appeared healthy on Nov. 22, 1957. At this time, one of these mice was killed and the blood serum was used for a dye test. The titer was 1:4096.

The other three mice were given a challenge inoculation intraperitoneally of approximately 20,000 RH Toxoplasma organisms. Three new mice were given inoculations at the same time of the same amount of the inoculum. These control mice all died in five days.

The protected mice began to look somewhat ill on the ninth day. One of them died on the 10th day, but Toxoplasma organisms were not seen in the small amount of peritoneal fluid obtained. The other two mice remained alive but showed definite post-encephalitic symptoms.

Guinea Pigs: Two guinea pigs with negative dye-test titers were given inoculations subcutaneously on April 17, 1957, of 0.1 ml. of peritoneal fluid withdrawn from mice of Subinoculation 5. They did not become obviously sick, but within two months they developed dye-test titers of 1:16,384. At present the titer of one remains at 16,384 and that of the other has dropped to 1:4096.

Two additional guinea pigs were given inoculations subcutaneously on Oct. 8, 1957, of 0.5 ml. of peritoneal exudate from mice of Subinoculation 10. After one month one has a dye-test titer of 1:16,384 and that of the other is 1:4096.

Rabbits: Two rabbits were given inoculations subcutaneously on May 10, 1957, of 0.1 ml. of undiluted peritoneal fluid from mice of Subinoculation 6. Neither became ill. One of them developed a dye-test titer of 1:16,384, but the other remained negative. Because of the difficulty encountered in obtaining peritoneal fluid from the mice at that time, it is possible that there were no organisms in the small amount of fluid inoculated into this rabbit. The rabbit which did not develop a titer and another new rabbit were given inoculations subcutaneously on Oct. 2, 1957, of 0.5 ml. of peritoneal fluid from mice of Subinoculation 10. Their dye-test titer now is 1:16,384.

Egg: Inoculation of 600,000 organisms of the stepped-up MF strain into chick embryo allantoic membranes caused marked

Fig. 11.—Chick allantoic membrane four days after receiving inoculation of the MF strain (see text).

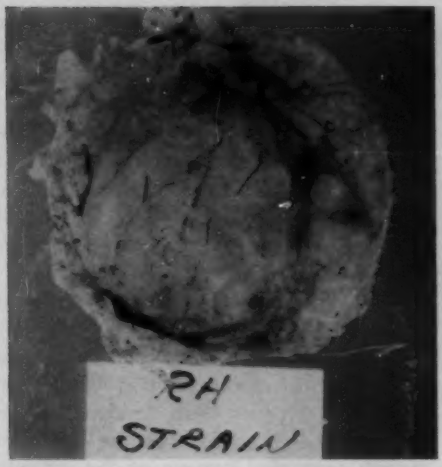


Fig. 12.—Chick allantoic membrane four days after reciving inoculation of the RH strain.

Fig. 13.—Chick allantoic membrane control; nutrient medium only was inoculated.

thickening and many pox. This greatly resembled the lesions caused by inoculation of 160,000 RH Toxoplasma organisms (Figs. 11, 12, and 13).

This strain offers a distinct contrast in virulence to that isolated and cultured from an eye by Jacobs and the strains obtained from severe systemic infections.

Comment

These studies have been of great interest to us because of our suspicion that some cases of inapparent congenital toxoplasmosis, with late exacerbations of the disease, are caused by Toxoplasma strains of low virulence.

This idea was suggested by the fact that in many cases of congenital toxoplasmosis the disease is extremely mild and is unrecognized until a late flare-up occurs. The present patient had a quiescent disease up to the age of 12, and the significance of the healed chorioretinitis which was discovered at the age of 1½ was not apparent to the physician or the family.

The isolation of Toxoplasma from the patient's eye 20 years after birth demonstrates the great persistence of these organisms in pseudocyst form in the tissues, despite the low virulence of the strain. The differences in characteristics between the RH and MF strains which are discussed point up the low virulence of the MF strain for animals.

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Some Intraocular and Conjunctival Effects of Amphotericin B in Man and in the Rabbit

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Intraocular mycotic infection is rare, and it usually necessitates enucleation of the affected eye. We recently encountered three instances of fulminating fungus endophthalmitis occurring as a postsurgical complication of cataract extraction, in which a pure culture of the Volutella species * was isolated from each of the infected eyes. We believe that these cases are the first of this kind to be reported. In two cases the infection failed to respond to conventional therapy and was terminated only by enucleation of the eye. In an effort to check the progress of infection in the third eye, a new antifungal antibiotic, amphotericin B,† was administered by parenteral, conjunctival, and intraocular routes. This clinical trial of amphotericin B for ocular infection, together with the experimental use of the antibiotic in the eyes of rabbits, is the subject of this report.

Amphotericin B is a polyene antifungal antibiotic which was isolated in 1955 by Gold, Stout, Pagano, and Donovick[1] from a soil strain of Streptomyces originating in Venezuela. In agar dilution tests, amphotericin B exhibited marked fungistatic action against numerous species of fungi, such as Candida albicans, Cryptococcus neoformans, Histoplasma capsulatum, Blastomyces dermatitidis, Blastomyces brasiliensis, and Sporotrichum schenckii. Higher degrees of resistance to the antibiotic were encountered with other pathogenic fungus species, such as Nocardia asteroides, Aspergillus fumigatus, Phialophora verrucosa, Hormodendrum compactum, and Hormodendrum pedrosoi.[1]

The pharmacology of amphotericin B in man and animals and the clinical uses of this antibiotic for the deep mycoses have been described.[1-14] The maximum intravenous dose of amphotericin B tolerated by humans is 1.0 to 1.5 mg. per kilogram per day, which produces a blood level of the antibiotic no greater than 1.8μg. per milliliter.[14] Although this level is higher than is necessary to inhibit most species of pathogenic fungi, it is too low to check the growth of the Volutella species, which requires a concentration of 10μg. per milliliter of amphotericin B. In view of these limitations, it seemed reasonable to employ direct intraocular injections of the drug to inhibit the growth of this fungus. In the absence of clinical data on the safety of intraocular administration of amphotericin B in humans, it was necessary to conduct preliminary studies of the clinical and pathological effects of such instillations in the eyes of experimental animals. Rabbits were chosen for this purpose because of their availability and relative ease of handling. The effect of amphotericin B, in varying intraocular

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* Dr. G. A. deVries, Centraalbureau voor Schimmelcultures, Baarn, Netherlands, provided the taxonomic identification of the culture. The closest species name that could be applied to this organism was Volutella cinerescens (Cesati) Sacc., belonging to the family Tuberculaceae.

† The trade name of E. R. Squibb & Sons, Division of Olin Mathieson Chemical Corporation, for amphotericin B is Fungizone.

and conjunctival dosages, was studied in healthy and in Volutella-species-infected rabbit eyes.

Methods

Amphotericin B Solutions.—Solutions of amphotericin B were prepared aseptically in sterile distilled water in concentrations varying from 500μg. to 5000μg. per milliliter. All intraocular doses of the drug used for both rabbit and human eyes were brought to a standard volume of 0.05 ml. Injections of amphotericin B solutions were made with a sterile 1 ml. tuberculin syringe and 26 or 27 gauge hypodermic needle into the anterior chamber of anesthetized human and rabbit eyes. The Van Lint anesthesia technique, employing retrobulbar injections of 2% procaine (Novocain), was used for the human eye, while ether anesthesia was used for rabbits. Single intraocular doses of amphotericin B in rabbits varied from 25μg. to 250μg. For topical use, amphotericin B was mixed in bacitracin ointment in concentrations varying from 250μg. to 50,000μg. per gram and applied daily.

Experimental Infections.—A washed saline suspension of the Volutella species was prepared from a four-day-old culture growth on Sabouraud dextrose agar. The suspension matched the turbidity of a Macfarland No. 1 standard and contained approximately 115,000 spores and mycelial segments per milliliter, as ascertained by direct microscopic count in a blood cell counting chamber. A culture suspension containing approximately 3500 spores was injected into the anterior chamber of each rabbit eye.

Before and after enucleation of the rabbit eyes, aqueous humor was aspirated and cultured for fungi on Sabouraud dextrose agar, liver-spleen glucose blood agar, and Littman oxgall agar. Cultures for bacteria were made in veal heart infusion blood agar and in thioglycollate broth. All enucleated eyes were fixed in 10% formalin, imbedded in celloidin, sectioned at 15μ to 30μ, and stained with hematoxylin and eosin and with Gridley fungus stains.

Experimental Study

The eyes of 20 female New Zealand white rabbits weighing 2 to 3 kg. were used for study. The animals were divided into three groups.

Group A. Drug Controls, Intraocular Injection.—The left eyes of nine rabbits were injected with a standard volume (0.05 ml.) of amphotericin B solution into the anterior chambers; the right eye of each animal served as a control. These single doses contained 25μg. to 250μg. of amphotericin B. Enucleations were performed 4 to 15 days later.

Clinical Changes: A single dose of 25μg. of amphotericin B caused slight hyperemia of the iris, opacity of the lens, and slight palpebral conjunctival congestion, all of which resolved in 36 hours with no clinical or pathological sequelae. Doses of 35μg. and 50μg. caused palpebral and bulbar conjunctival congestion, corneal edema, slight clouding of the aqueous humor, iridal hyperemia, and opacity of the lens. All of these effects resolved in 96 hours, with no clinical or pathological sequelae. Doses of 125μg. and 250μg., on the other hand, caused severe chemosis of the bulbar and palpebral conjunctivae, marked corneal edema, and clouding and exudation in the anterior chamber. Most of these changes did not resolve. The results are summarized in Table 1. Cultures of all eyes in this group were negative for bacteria and fungi.

Group B. Drug Controls, Conjunctival Application.—Conjunctival applications of bacitracin ointment containing amphotericin B in concentrations of 250μg. to 50,000μg. per gram were made twice daily for four days to the eyes of four rabbits. Enucleations were performed 7 to 11 days later. Cultures of all eyes were negative for bacteria or fungi.

Clinical Changes: Doses up to and including 500μg. per gram were well tolerated and failed to cause an irritating effect in the rabbit eye. Higher concentrations, up to 50,000μg. of amphotericin B per gram, caused a slight palpebral conjunctival con-

TABLE 1.—*Summary of Clinical Changes Observed in Rabbit Eyes Injected with Single Doses of Amphotericin B*

Dose, μg.	Conjunctiva Congestion	Cornea Reaction *	Anterior Chamber Clouding & Exudation	Iris Hyperemia	Lens Opacities	Fundus Reflex	Resolution
25	+	0	0	+	+	+++	Complete, 36 hr.
35	++	+	+	+	+	+++	Complete, 96 hr.
50	++	+	+	++	++	++	Complete, 96 hr.
125	+++ Chemosis	++	++	++	Not Visualized	+	Incomplete; corneal opacity & pannus, posterior synechiae
250	++++ Chemosis	+++	+++	+++	Not Visualized	0	Incomplete; corneal opacity & pannus, posterior synechiae

* Edema, clouding, and pannus.

gestion, slight epithelial edema of the cornea, and iridal congestion. These changes resolved within six days.

Group C. Volutella-Species-Infected Eyes Treated Intraocularly with Amphotericin B.—Seven rabbits (13 eyes) received injections of spore suspension into the anterior chamber. As shown in Table 2, some of these eyes were treated intraocularly with one or two doses of amphotericin B, administered over varying periods of time, while the other eyes remained untreated. Three successive intraocular doses of 25μg. to 35μg. of amphotericin B, every three days, produced reactions similar to those following a single dose, with resolution each time.

Clinical Changes: Injections of fungus spore suspensions caused clinical changes in the rabbit eye similar to those produced by 50μg. of amphotericin B alone (Table 1). These changes resolved within six days, while those produced by amphotericin B alone took only four days (Table 1). The initial reaction in the anterior chamber to the drug or spore suspension appeared to be due to trauma, to the introduction of foreign matter, or to both.

There were only four established infections in 13 rabbit eyes inoculated with Vol-

TABLE 2.—*Summary of Results in Volutella-Species-Infected Rabbit Eyes Treated Intraocularly with Amphotericin B*

Rabbit		Intraocular Therapy				Enucleation	Culture		Histological Findings	
No.	Eye	Day *	Dose, μg.	Day *	Dose, μg.	Day *	Days *	Fungus Isolation	Inflammation	Fungus
5	O. D.	6	25	8	125	29	23, 29	0	0	0
	O. S.	6	25	8	50	29	23, 29	0	0	0
6	O. D.	6	30	8	30	79	23, 79	0	0	0
	O. S.	6	15	8	45	79	79	0	0	0
7	O. D.	2	25	8	35	79	23, 79	0	0	0
	O. S.	8	40	—	—	79	23, 79	0	0	0
8	O. D.	—	None	—	—	74	18	0		
							74	+	Abscess	+
	O. S.	—	None	—	—	74	18, 74	0	0	0
18	O. D.	—	None	—	—	60	4, 60	0	0	0
	O. S.	—	None	—	—	60	4, 60	0	0	0
19	O. D.	24	40	40	35	60	4, 60	+	Abscess	+
	O. S.	No fungus injected				60	60	0	0	0
20	O. D.	—	None	—	—	60	4	0		
							60	+	Abscess	?
	O. S.	24	125 (topical)			60	4	+		
							60	0	Organized	0

* Day after injection of fungus suspension.

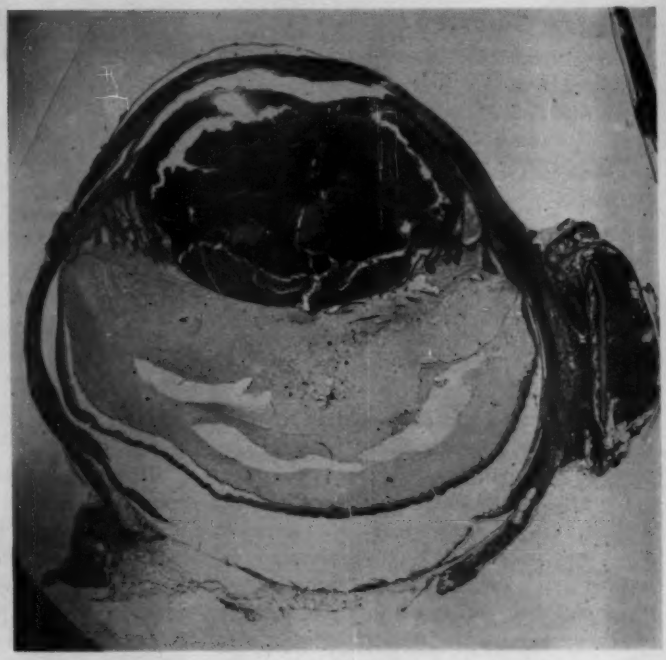


Fig. 1 (Rabbit 19).—Right eye. Note the anterior chamber abscess, the massive lenticular detruction, and the ciliary involvement. Hematoxylin and eosin; × 6.

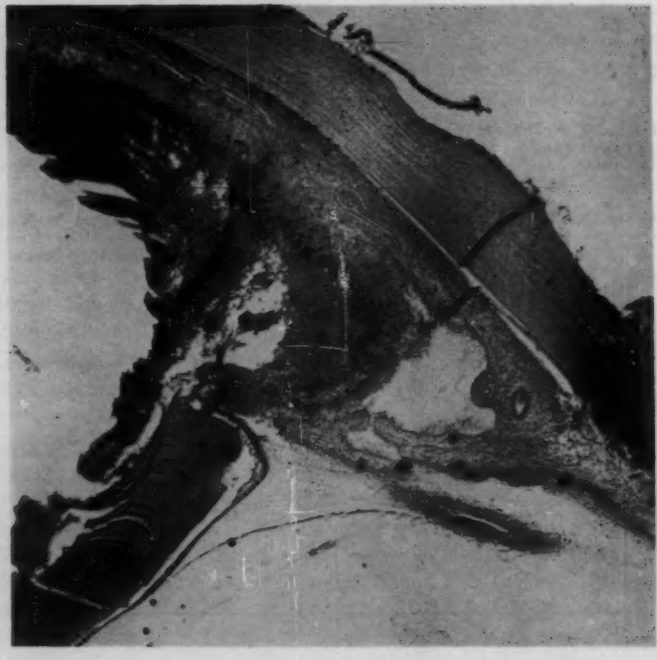


Fig. 2 (Rabbit 20).—Right eye. The destructive process is similar to, but less advanced than, that seen in Figure 1. Note the leukocytes passing through the zonular ligaments. Hematoxylin and eosin; × 22.

Fig. 3 (Rabbit 20).—Left eye. The pupil is obliterated by hyalinized fiber adhering to the corneal endothelium. Hematoxylin and eosin; × 22.

Fig. 4 (Rabbit 19).—Right eye. Fungal hyphae radiating from a colony near the edge of the abscess. Gridley stain; × 1000.

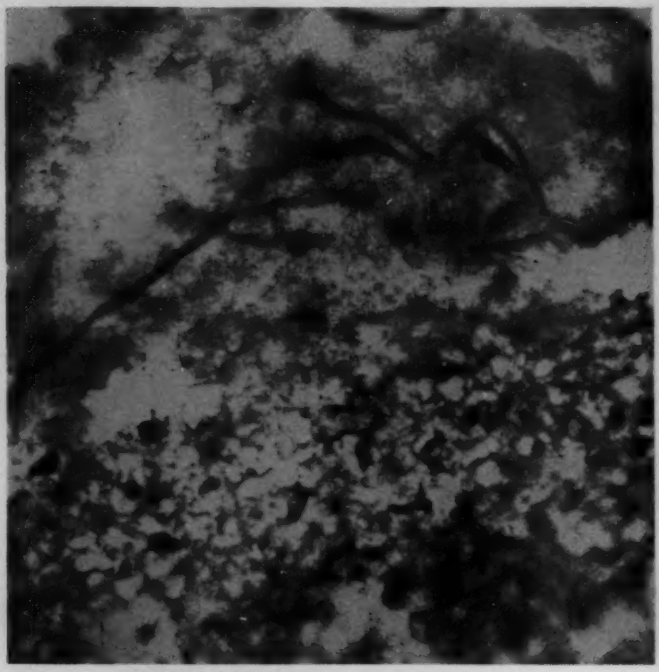


Fig. 5 (Rabbit 8).—Right eye. Fungal hyphae at edge of abscess. Gridley stain; × 1000.

utella species spores, while all others resolved. This low incidence of infection, despite the introduction of approximately 3500 fungus spores in each eye, was attributed to the low virulence of the organism coupled with high tissue and/or host resistance. There was at least one instance of established infection in which spontaneous cure occurred without treatment, viz., the left eye of Rabbit 20 (Table 2). One may discount the single, late conjunctival dose of amphotericin B given in this case.

The treatment schedule, culture results, and histological findings in the rabbit eyes

Fig. 6 (Rabbit 20).—Right eye. Macrophage filled with fragmented PAS-positive matter. Gridley stain; × 1800. Slightly retouched.

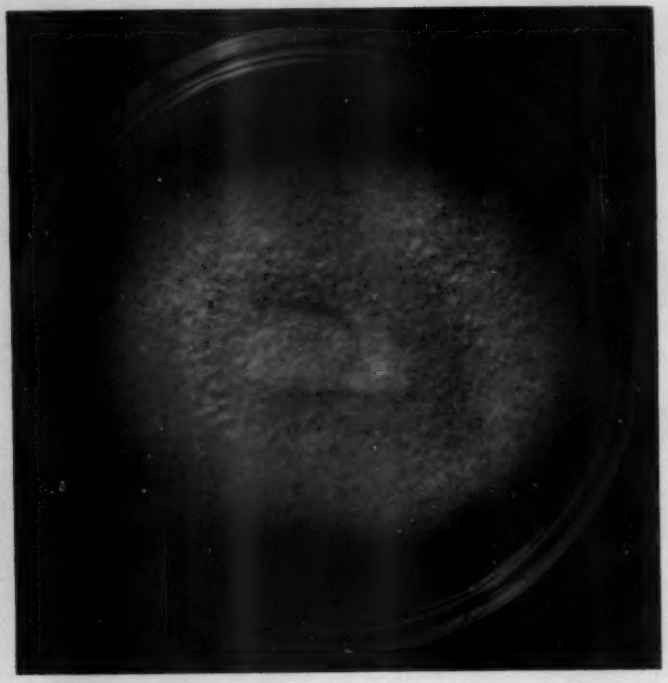


Fig. 7.—Sporophores and conidia of Volutella species in microculture. Although the resemblance to Cephalosporium species is striking, this type of spore formation is secondary to the production of sporodochia or fruiting bodies of interwoven conidiophores (not shown here) which are characteristic for Volutella; Slide culture; unstained; ×2000. Slightly retouched.

Fig. 8.—Giant filamentous colony of Volutella species on Sabouraud's dextrose agar at 20 C. ×1.5.

of Group C are summarized in Table 2. Within 6 to 10 days after fungus inoculation infection of the eye was characterized by the appearance in the anterior chamber of macroscopic creamy nodules which measured 0.1 to 0.2 mm. in diameter and had sparse gray filamentous edges. The clinical courses of the four established infections varied as follows:

RABBIT 8.—Infected right eye. No treatment. Growth of the fungus was minimal, disappearing by the 21st day, and had not recurred at the time of enucleation 53 days later.

RABBIT 19.—Infected right eye. Two intraocular doses of amphotericin B (40μg. and 35μg.). Destruction of the structures of the eye was progressive. A hypopyon obliterated the anterior chamber, and mild buphthalmos was observed.

RABBIT 20.—Infected right eye. No treatment. A well-demarcated oval iridal exudate, measuring 1×4 mm., developed at 4 o'clock, and the pupil was pulled upward.

RABBIT 20.—Infected left eye. Conjunctival application of amphotericin B. A hypopyon developed, disappearing on the 30th day. This was replaced by an exudate similar to that seen in the right eye. The pupil became an eccentric yellowish dot located at 10 o'clock.

Pathological Changes: Successful experimental infection of the anterior chamber with the Volutella species was characterized by abscess formation, keratitis, corneal necrosis, iritis, lenticular destruction, and eventual involvement of the vitreous. The abscess was comprised of polymorphonuclear leukocytes, round cells, and monocytes. Gridley fungus stains revealed fungal hyphae near the periphery of the abscess, but hematoxylin and eosin stains had failed to demonstrate them. When healing occurred, it was by the process of granulation and fibrosis, with the clinical sequelae dependent on the formation of adhesions. The killed fungus was undoubtedly removed by phagocytosis. The right eye of Rabbit 20 yielded a positive culture at enucleation, and no hyphae were observed in or near the abscess with Gridley stain. However, numerous macrophages contained periodic acid-Schiff (PAS)-positive fragments which were interpreted as products of fungus cells and fungus "dust." In contrast, the left eye of Rabbit 20, which yielded a positive culture at four days but was sterile at enucleation, contained an organized exudate in the anterior chamber. This area, however, was devoid of PAS-positive fragments.

Clinical Study

A 78-year-old white woman with bilateral nuclear cataracts underwent first right and then left intracapsular cataract extractions. Endophthalmitis appeared in the right eye nine days after the first operation. Despite intensive treatment with nystatin (Mycostatin) orally and locally, chloramphenicol (Chloromycetin) orally, and penicillin intramuscularly, the endophthalmitis increased in severity, necessitating enucleation of the eye on the 47th postoperative day. Culture examination of the eye yielded a pure culture of Volutella species, and histological

Color Plate.—Effects of single intraocular injections of amphotericin B in rabbit and human eyes.

A-G, Controls, rabbit eyes:

A, 25μg. Bulbar and palpebral conjunctival congestion. Resolution in 36 hours.

B, 35μg. Bulbar and palpebral conjunctival congestion and chemosis, slight corneal edema, and minimal exudates in the anterior chamber; iris hyperemia. Resolution in 96 hours.

C, 125μg. Moderate bulbar and palpebral conjunctival congestion and chemosis, moderate corneal edema, and exudates in the pupillary area. Iris hyperemia. Resolution incomplete.

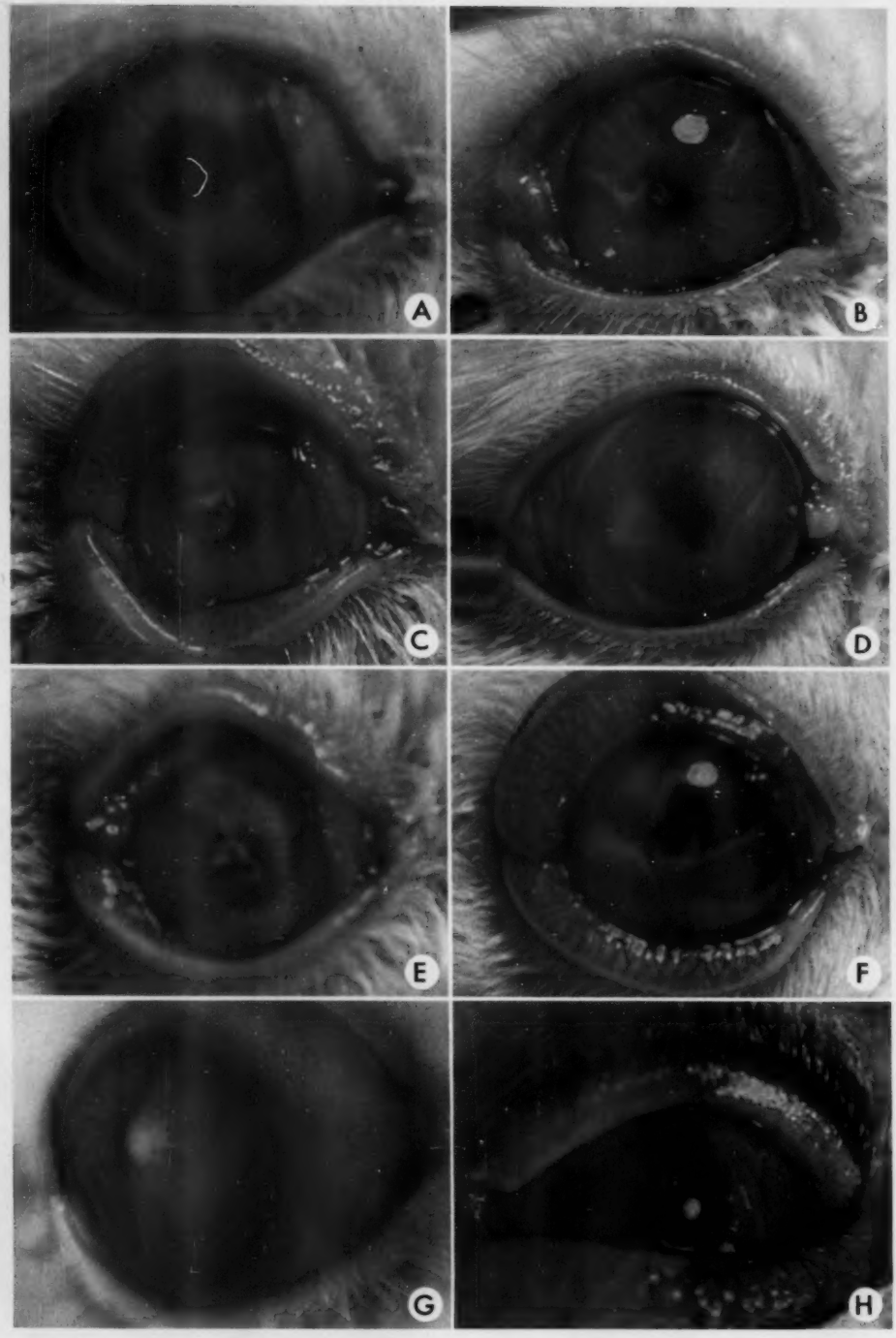
D, 250μg. Severe bulbar and palpebral conjunctival congestion and chemosis; severe corneal edema and staphyloma. Exudates in anterior chamber and iris hyperemia. Resolution incomplete.

E, Rabbit 5, O. D. (Table 2), six days after 25μg. of amphotericin B. Slight bulbar and palpebral conjunctival congestion. No remarkable absorption of the grayish exudate in the anterior chamber. Resolution clinically after 29 days.

F, Rabbit 6, O. D. (Table 2), six days after 30μg. of amphotericin B. Slight bulbar and palpebral conjunctival congestion and hyperemia. Corneal edema and pannus between 9 and 12 o'clock. Clouding of aqueous. Resolution after 29 days.

G, Rabbit 20, O. D. (Table 2), 60 days without treatment. Buphthalmos, severe conjunctival congestion and chemosis, corneal opacity, abscess in the anterior chamber. Severe iris hyperemia.

H, Patient's left eye after two doses of amphotericin B. Bulbar and palpebral conjunctival congestion, cornea clear, receding of fungus growth.


A
B
C
D
E
F
G
H

examination revealed an anterior chamber abscess in which numerous spores and branching hyphae of the fungus could be demonstrated with Gridley stain but not with hematoxylin-and-eosin stain.

Early signs of endophthalmitis also appeared in the left eye, 31 days after intracapsular extraction of a cataract. Three gray nodules, averaging 0.5 mm. in size, appeared on the anterior surface of the vitreous near the pupillary border of the iris, at 6 o'clock. Gray filaments extended radially about each central nodule.

Thirty-six days after operation, 75 mg. of amphotericin B in 500 cc. of 5% dextrose in water for injection U. S. P. was administered intravenously over a period of six hours. This dose was repeated on alternate days, without effect on the ocular disease. Intravenous therapy was discontinued when the minimal inhibitory concentration of amphotericin B for the Volutella species was found to be 10μg. per milliliter, or approximately five times the maximal blood level of amphotericin B (1.8μg. per milliliter).

Guided by the tolerances of rabbit eyes to amphotericin B, as described above, and with the use of local procaine anesthesia, we administered this antibiotic in 0.05 ml. volumes. It was given intraocularly, subconjunctivally, and conjunctivally (in an ointment) as follows:

Postoperative Day	Dose of Amphotericin B, μg.
43	12.5 intraocularly (anterior chamber)
46	35 intraocularly 15 subconjunctivally 5000 per gram of bacitracin ointment, conjunctivally, daily for 5 days
55	35 intraocularly 15 subconjunctivally 5000 per gram of bacitracin ointment, conjunctivally, daily for 5 days
75	40 intraocularly

Each intraocular injection of amphotericin B into the anterior chamber produced an exudation similar to that observed in the rabbit eyes. Within 48 hours the exudate began to diminish, leaving the pupil covered with a yellowish membrane. As the edges of this membrane receded, the fungus nodules enveloped within the membrane began to decrease in size, and their gray radial filaments disappeared entirely. Clinical improvement was noted to be greatest by the 10th day after the first injection and by the 7th day after succeeding injections. By the 50th postoperative day vision was limited to counting fingers at 6 ft. with a +10 lens. Fungus nodules were no longer visible. The fungus growth in the eye recurred, however, within 12 days, and a final injection of 40μg. of amphotericin B was given. The outcome was a pannus, hammock pupil, and decreased intraocular tension.

By the 111th postoperative day the left eye was clinically "quiet." On the 132d postoperative day central iridotomy revealed a contracted vitreous and a funnel-shaped retinal detachment but no active infection. At the present time, 15 months after the original operation, the patient has an asymptomatic, fungus-free, but blind eye.

Summary and Conclusions

Amphotericin B, administered by intraocular, subconjunctival, and conjunctival routes in a human case of Volutella species infection of the anterior chamber of the eye, seemed to eradicate the fungus. However, sufficient damage had occurred during the course of infection to produce a pannus, hammock pupil, contracted vitreous, retinal detachment, and blindness.

The safe intraocular dosage of amphotericin B in the rabbit and human eye is 20μg. to 35μg. injected into the anterior chamber in a volume of 0.05 ml. every 10 days for a total of three doses.

A safe range of concentration of amphotericin B for daily application to the rabbit and human conjunctiva and cornea is 5 to 10 mg. (5000μg. to 10,000μg.) per gram of bacitracin ointment.

Infection of the rabbit eye with Volutella species is difficult, even with a heavy inoculum. Once induced, infection with the fungus species may proceed to panophthalmitis or may regress spontaneously.

The effects of amphotericin B in the healthy and Volutella-species-infected rabbit eye and its application to a case of Volutella species endophthalmitis in a 78-year-old woman, which occurred after an intracapsular cataract extraction, are described. A course of therapy such as that described may prove helpful in other fungus endophthalmitides if instituted promptly, particularly if the invading organism is more sensitive to amphotericin B than is Volutella species.

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The Character of the B-Wave in the Human Electroretinogram

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Introduction

It is now nearly 100 years since Frithiof Holmgren placed recording electrodes on an eye and discovered that a momentary change in potential accompanies the reaction of the eye to light. The electrical response that he found paralleled subjective aspects of vision in several ways. Research since Holmgren's time has been directed toward fuller exploration of these parallels.

The electroretinogram, or ERG, has proved to be a complex phenomenon. The search still continues for ways in which aspects of the responses may be used as objective indices of visual processes. For the physiologist this electrical behavior is interesting both for itself and for what it reveals of neural activity within the eye. To the psychologist there occurs the hope that it may enable him to bypass some of the tortuous paths of psychophysics. And to the clinician the ERG presents itself as a possible means of diagnosing particular functional deviations. The purposes of all these will be best served if the attention of experimenters can be centered on the task of producing an accurate, detailed picture of the forms and variations of response correlated with major stimulus variables. Not until these various parameters have been adequately cataloged will we be able to select the most suitable conditions for observing particular aspects of the response or to suggest standard conditions for clinical use.

Much of the interest in the human ERG, whether supported by practical-clinical or by theoretical-experimental considerations, has centered on the b-wave, the most prominent component of the response. In this paper I shall try to set forth, in organized fashion, what is known of the b-wave. The presentation will be selective rather than exhaustive, its content and emphasis determined by the concept of research need outlined in the previous paragraph. To material available in the literature I shall add some new facts and some new interpretations which have emerged incidental to work carried out in the psychological laboratory of Brown University during the past several years.

The Phenomenon as First Viewed.—In 1865 Holmgren [45] reported a momentary increase in the electrical positivity of the cornea at the onset of light. The phenomenon did not immediately command the attention of the scientific world, and no one other than Holmgren did anything with it until its independent rediscovery in 1873 by Dewar and McKendrick.[30-32] These investigators [31] carefully bisected the eye of a frog, so as to remove completely the anterior portion, including cornea, iris, ciliary muscle, and lens. With the retina thus isolated they found the same response, as sensitive to light as when the whole eye was employed. They demonstrated that it was the retina, and not some other portion of the eye, which was electrically responsive to stimulation by light. They went on to study the response in a variety of animals, including man. With the introduction of new recording techniques, around the turn of the century, interest developed in intricacies of the response previously overlooked or disregarded. But before investigation graduated to this new plane of concern for

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individual "components" in the response several significant observations had been made, primarily relevant to the major component, or, as we usually know it today, the b-wave.

A comparison of electrical responses from the eyes of experimental animals with some of the well-known aspects of human vision revealed significant parallels. Thus, Dewar and McKendrick[31] had found the amplitude of the electrical response roughly proportional to the logarithm of the stimulus intensity—suggestively in agreement with Weber's law. Kühne and Steiner[55] had observed that adaptation to light reduced the magnitude of the response while dark adaptation restored it. Dewar and McKendrick[32] and Holmgren himself[46] had seen that the middle wavelengths, those which appear brightest to the human eye, were also most effective in evoking electrical responses. The relationship, or at least the relevance, of the ERG to visual processes was well established.

Analysis of the Response into Components.—The first discoverers remarked only the presence of positive* on- and off-responses, as in the first diagram (Fig. 1*A*). But in 1903 Gotch,[37] using a capillary electrometer, obtained records without serious distortion, and in these he noted a small negative deflection just ahead of the main positive on-response. A second, slow or late, positive component also began to be noted, though this was attributed by some to muscle artifact associated with the pupillary response. Brücke and Garten,[22] using a string galvanometer, soon reaffirmed the retinal origin of the slower component.† They also confirmed the existence of Gotch's early negative component. In their work they demonstrated the basic similarity of responses produced by animals throughout the vertebrate series, from fish to monkey. Though latency and duration varied from species to species, the typical response was seen as follows: first a brief negative dip, immediately succeeded by a larger positive swing which soon declined somewhat and then either remained level or rose again slightly (second positive wave) until the light was turned off. At "off" there was another small positive swing, then a return of the potential to the base line. A conventionalized drawing of the waves described here appears in Figure 1*B*.

The fashion of referring to the various parts of the response by letter dates from 1908, when Einthoven and Jolly[35] presented their historic analysis. They show records from the frog eye, responses to lights varying in intensity and in duration. On

* As the response is typically recorded with the active electrode on the cornea, a positive component is one characterized by positivity of the cornea with respect to the fundus.

† Even though, in work with animals, such responses have appeared after the iris has been removed[24] or paralyzed,[22] iris artifacts are occasionally taken for retinal responses. Karpe[27] has demonstrated clearly the appearance of this artifact in the human ERG.

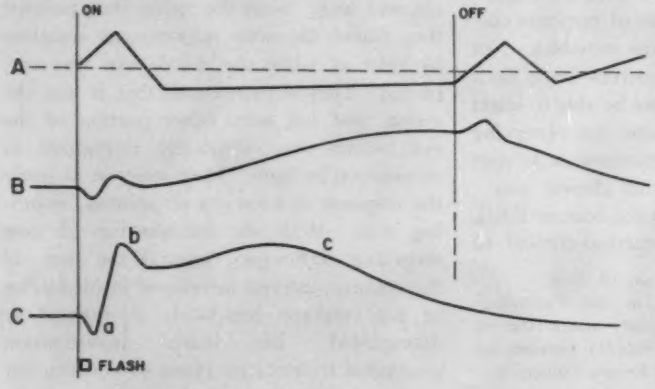


Fig. 1.—*A*, from a figure published by Dewar and McKendrick.[31] A diagrammatic reconstruction of galvanometer deflections which the authors observed as the stimulus was turned on and off. *B*, diagrammatic representation of the ERG as observed and described by Brücke and Garten[22] and by other early investigators. On- and off-responses shown. *C*, the response to a brief stimulus flash with *a*-, *b*-, and *c*-waves designated. After a figure presented by Einthoven and Jolly.[35]

these records the observed peaks are labelled as a-, b-, and c-waves. These letter-designations are shown in Figure 1*C*. A positive off-response at the cessation of long flashes of light (somewhat longer than that chosen for illustration) was called by them the a_1-wave but is now regularly known as the d-wave.

Three underlying processes were assumed by Einthoven and Jolly:

(1) A response of short latency, negative to light, positive to darkness (the a- and d-waves were its manifestation).

(2) A response of longer latency, positive to light (the b-wave), more sluggishly negative to dark.

(3) A very sluggish response, positive to light (the c-wave) and negative to dark.

These components are diagrammed in Figure 2*A*. Little justification has ever been advanced for the inclusion of off-responses as parts of the second and third processes. Much of the behavior of the response *was* made more intelligible, however, by viewing the response as the algebraic summation of independently varying processes.

Waller[72] published his analysis in 1909. As he was an "unbeliever" in the matter of the c-wave, he proposed two rather than three components. He chose to portray these, however, in a rather different manner. His a-, b-, and d-waves appeared as resultants of the interplay between a positive component, which accounted for the b-wave, and a negative component of shorter latency (Fig. 2*B*). In his reasoning Waller made reference to the negative response recorded from aging (excised) eyes to establish the form of the negative component. A more prominent resultant b-wave could, of course, have been provided by Waller either by creating his positive component with an initial bump or by supposing that the negative component, despite its shorter latency, developed to its maximum more slowly. Piper,[60] whose analysis is shown

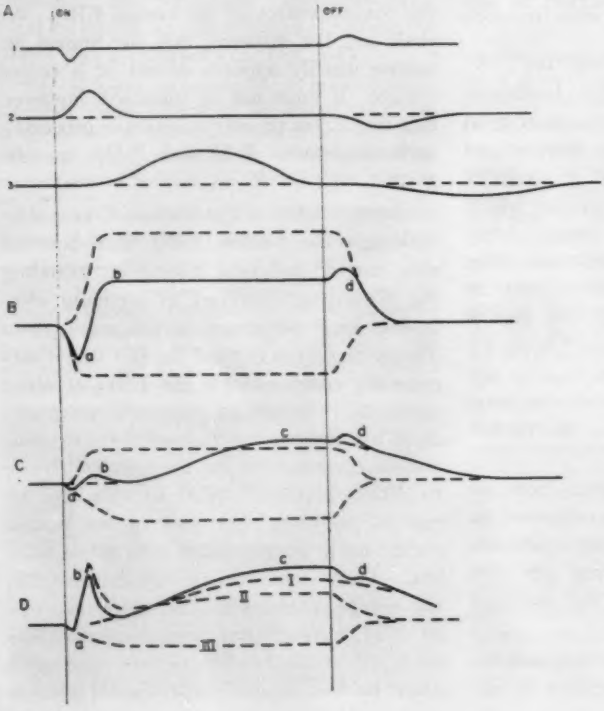


Fig. 2.—Components of the ERG according to the analyses of the following authors: *A*, Einthoven and Jolly; *B*, Waller; *C*, Piper; *D*, Granit.

in Figure 2*C*, made the latter choice, while Granit,[39] some years later, chose to combine the two. Granit's version appears in Figure 2*D*. The general similarity of Granit's analysis to that of Piper may be seen. The merit of Granit's solution lay in the fact that he could offer evidence for the selective effect of certain procedures (altering the condition of the organism) on each of his proposed components. Specifically, he found that when ether was administered the first change was a diminution of the slow positive component (c-wave, P-I), leaving a positive remainder. This remainder then diminished progressively until only the negative response (P-III) was left. With prolonged anesthesia this, too, disappeared. When the carotid artery was occluded, P-II appeared to be selectively eliminated. In this case the response appeared as an initial large negative deflection (P-III) which passed smoothly into the still larger positive c-wave (P-I). The off-effect (d-wave) showed up when P-III returned rapidly to the base line. Responses observed in this work were those of the cat.

If we may disregard the negative "off" phase tentatively suggested by Einthoven and Jolly, all of these analyses are in good agreement with respect to the b-wave and the process underlying it (e. g., Granit's P-II). It is a swing toward positivity which occurs on illumination of the retina. After an initial latency it begins and rises smoothly to a maximum. Following this it may or may not decline. The matter of this decline need not be considered an issue, for, as we shall see later, whether it does or does not diminish in this fashion depends (at least in the case of the human ERG) on the circumstances of stimulation.

Various other qualitative observations on the behavior of the b-wave as observed in work on animals under different conditions might be listed. The following are well known and may be found in the standard reviews.[40,41,54,64]

The b-wave may appear in response to either short or long flashes. It may be enhanced by administration of glucose but is easily depressed by drugs. It is first excited, then depressed, by strychnine. Potassium or calcium will obliterate it—with an increased latency of response accompanying its progressive diminution. Epinephrine has a similar effect. Therman[69] and, more recently, Noell[59] have contributed largely to our knowledge of the effects of chemical agents.

Continuing, we note that both amplitude and latency of response are greater in the dark-adapted eye. An increase in either the intensity or the area of the stimulus may decrease the latency and increase the magnitude of the response, within limits. The b-wave is now generally believed to originate in or near the rod-cone layer of the retina.[20,59]

This brief historical résumé does not exhaust the topic of "components." But further discussion will be restricted to components of the human response and may best be postponed until we have run through the characteristics of the human ERG. We shall see, for instance, that the human response usually appears devoid of a retinal c-wave. It must not be supposed, however, that this leaves us only two simple processes, such as Granit's P-II and P-III, to integrate.

Characteristics of the Human Response.—Although the human ERG was detected very early,[29] technical difficulties, including the discomfort involved in applying electrodes, long prevented investigators from discovering much beyond the fact that it was generally comparable to the ERG of other mammals. Ambitious research programs dwindled away in the face of the difficulties and uncertainties of the methods employed. In 1925 Hartline[44] tried to simplify the task of applying electrodes to the human eye by using goggles filled with saline solution. These were reasonably comfortable, but muscle artifacts spoiled many records. In 1941 Riggs[62] described a contact-lens electrode which he had devised and with which he had obtained reproducible records

of excellent quality. Some such device is now employed in most human ERG work. It did not come into general use immediately, however, and in 1944 Adrian [1,2] made some very significant observations on the human ERG without its aid. He used a moist thread on the cornea of his own eye and succeeded in obtaining records under several different conditions. These were free of the grosser artifacts which had plagued his predecessors, and, though his recording device used a resistance-capacitance coupled amplifier and an ink-writer, distorting the form of the response slightly, his results were unambiguous.

Adrian used brief (1/30 second) flashes of light as stimuli. His most striking discovery was that two distinct b-wave components appeared ‡ when orange stimuli were presented to the dark-adapted eye. Red light elicited only the component with the shorter latency; blue light, only the longer. Adrian, accordingly, assigned various portions of the response to photopic or scotopic processes. The picture of the human electrical response as described by Adrian in 1945 [2] was as follows (cf. Fig. 3*A*):

"The photopic component is a brief diphasic potential change. There is an initial negativity of the cornea . . . followed by a brief positive wave. The response is produced by white light or by monochromatic light of all colors except blue. It is present in the fully light-adapted eye and is not much increased by dark adaptation.

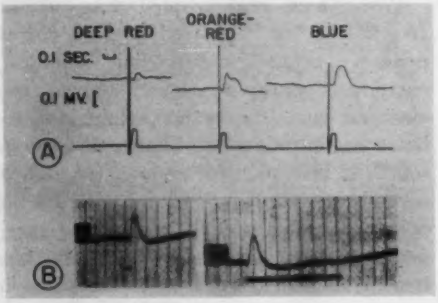
"The scotopic component is a slower monophasic change reaching 0.3-0.4 mV. with the cornea positive. It is produced by light of all colors except deep red. It is absent in the light adapted eye and is greatly increased by dark adaptation."

It was the earlier positive component that was "new." The negative a-wave had been seen previously in human as well as in animal records, and the positive "scotopic" component elicited with blue light in the dark-adapted eye was the familiar large b-wave previously noted in human ERG's. Its latency of a bit under 100 milliseconds, its maximum amplitude of 300 μv. to 400μv., reached about 150 milliseconds after the beginning of stimulation, conformed with the scattered observations of earlier investigators.§

Technically better records were presented by Karpe [52] in 1945, though his selection of stimuli precluded observation of Adrian's dual responses. He used a contact lens similar to that described by Riggs,[62] ‖ which he was able to apply, though not without attendant difficulties, to the eyes of patients

‡ B-wave complications had been reported in animals since the time of Gotch.[27]

Fig. 3.—*A*, human responses published by Adrian.[2] Photopic (response to red flash), mixed (orange-red), and scotopic (blue) records, *B*, responses recorded by Karpe,[52] using short (40-millisecond) and long (800-millisecond) stimulus flashes. Signal indicates 100-millisecond intervals.



§ The discovery of two positive components has led to a confusion in nomenclature. Adrian refers to the earlier merely as the postive phase of the photopic response. Granit,[40] in discussing Adrian's results, refers to both positive components as b-waves, one photopic and one scotopic. Motokawa and Mita,[57] however, in a prior, independent discovery of the photopic component, dubbed it the "x-wave." This designation has been taken up by some other experimenters, but a sampling of recent literature shows no clear decision regarding terminology. It would seem to me that Granit's instincts in the matter were sound. It would be preferable to refer to the two as photopic and scotopic b-waves, perhaps abbreviated as the b_p-wave and b_s-wave. This would permit the noncommittal designation, b-wave, to indicate either uncertainty or indifference to the distinction. Certainly in the past, particularly in work with animals, the term b-wave has been used thus to allude indiscriminately to photopic responses, scotopic responses, or admixtures of the two. Recent work [9,13,16] has indicated that the a-wave has a similar dual nature. Here again, where possible, differentiation might be made between the photopic a_p-wave and the scotopic a_s-wave.

‖ Karpe's electrode, though similar in principle to that of Riggs, was independently conceived.

in a clinic. He used a direct-coupled amplifier which recorded photographically on a moving tape. He provided an invaluable picture of the range of normal variation in the ERG under one standard set of conditions (20 lux of white light in an 8 degree stimulus-field, $\frac{1}{25}$-second exposure). These conditions are such as to elicit from most subjects a near-maximum b-wave with little or no a-wave. Amplitude of response varies from 230μv. to 510μv., with a mean of about 350μv. Latencies ranged from 30 to 60 milliseconds, 40 milliseconds being typical.¶ Karpe's extensive monograph is also valuable for its clear descriptions of various kinds of recording artifacts and for its depictions of the response under pathological conditions. A pair of Karpe's records (Fig. 3*B*) shows that prolongation of the stimulus to 800 milliseconds produces little or no change either in the amplitude or in the form of the response. Under other conditions, however, as we shall see later, the duration of the stimulus may be an important factor in determining the form of the response.

Quantitative Studies of the Human Response

With the foregoing material as an introduction, I shall attempt a systematic exposition of the effects of stimulation and adaptation variables on the b-wave. Since many of the studies to be described were carried out in the psychology laboratories of Brown University, it is appropriate to insert a brief, generalized description of the type of apparatus characteristically used.

The subject sits in an electrically shielded light-proof room. The active electrode is mounted in an individually fitted contact lens.# A neutral electrode, on the forehead, is held in place by an elastic headband. The leads pass to a high-gain direct-coupled amplifier that actuates a loop oscillograph or cathode-ray oscillograph with the excursions photographically recorded on moving film, along with time-interval and stimulus indicators. For stimulation, light from a tungsten filament, run at a known color temperature, passes through high-speed shutters, calibrated neutral and narrow-band filters, and optics to the eye. Experiments have been carried out with use of the so-called Maxwellian view, in which the light enters the eye as a pencil of rays focused at the pupil, and also with conventionally illuminated stimulus areas. The position of the subject's head and eye are maintained through the use of a biting board and fixation guides.

In my review of quantitative work the factors with which I shall be most concerned are (1) the spectral composition of the stimulus, (2) the intensity of the stimulus, (3) the duration of the stimulus, (4) the locus and the area or extent of the stimulus, and (5) the state of adaptation of the eye. Response measures most frequently met are those of amplitude (at peak), latency, and implicit time—the time from the beginning of the stimulus to the peak of the response.

(1) *Spectral Composition of the Stimulus.*—From almost the start [32,46] it was recognized that the size of the response was at least roughly related to the spectral sensitivity of the eye. For the large responses of the dark-adapted eye a scotopic function was conceived, with the possibility of a Purkinje shift.

Work with dark-adapted eyes of the frog [25] and rat [38] showed a close correspondence between the scotopic luminosity curve and "sensitivity curves" derived from electrical data. The method of obtaining the latter was introduced by Chaffee and Hampson.[25] It involved determining, for each of several stimulus colors, the amount of energy required to elicit a b-wave of specified amplitude. This energy figure was then treated as similar to a threshold value in

¶ It should be noted that Karpe did not regularly employ the condition of full dark adaptation in his studies.

A detailed description of the corneal electrode may be found in papers by Riggs.[42,43] The fullest description of the apparatus used in our earliest studies, and used with but minor modifications in much of the later work, is in a paper by Johnson.[47]

computing the electrical sensitivity curve.*

Granit and co-workers [42,43] confirmed the Purkinje shift in the frog eye, with a b-wave maximum at about 510 mμ in the dark-adapted eye and about 560 mμ in the light-adapted eye.

Clear differentiation of photopic and scotopic components in the b-wave of the human ERG was made by Adrian.[1,2] From his work the implication was clear that the early positive component, elicited by red or orange light, was photopic. The second and larger, appearing in response to all colors save deep red, was scotopic. Throughout the rest of this paper we shall refer to these two components as the photopic b-wave (b_p-wave) and the scotopic b-wave (b_s-wave), respectively, and to both as b-waves. (See discussion in the footnote in the right-hand column of p. 566.)

Riggs and Johnson,[66] using narrow-band filters, determined the relative effectiveness of lights of different wavelengths in eliciting the human b_s-wave and compared the results with psychophysical data. They used a 58μv. response criterion in the electrical study and scotopic matching in the psychophysical work. Both sets of data showed sensitivity peaks at about 500 mμ, but the electrical response data were elevated, indicating relatively greater sensitivity, throughout the blue end of the spectrum. More precise determinations by Riggs, Berry, and Wayner [65] confirmed these findings (Fig. 4*A*).

Boynton [18] suggested an explanation of the excess blue-sensitivity. He attributed it to stray light, reviving an earlier suggestion of Fry and Bartley [36] that when small stimulus areas are used (e. g., 7 degrees 30 minutes diameter in the studies just described) much of the response is to light scattered over the peripheral retina, outside the focal area. Since Rayleigh scatter varies with wavelength, blue would be scattered more than red and would effectively stimulate more of the retina. Boynton reasoned that this differential should be reduced if the "focal" portion of the stimulus is larger. He used larger areas and found the expected convergence toward normal scotopic values. Work in this laboratory has shown that with full-retinal stimulation sensitivity curves for the b-wave of the dark-adapted eye (b_s-waves) reproduce the scotopic luminosity function exactly. One may reasonably conclude that, even though the stimuli may be very intense—well above the photochromatic interval—this component of the ERG reflects activity of the scotopic visual mechanism.†

The b_p-wave has also been studied. A history of work on this wave is a history of

* Though this procedure appears straightforward, the concept of electrically determined "sensitivity" needs to be considered for a moment. Psychophysical functions (visibility curves, dark-adaptation curves, etc.) are determined either by measuring variations in the subjective light-threshold with varying conditions or by stimulus-equation techniques. We look for some sort of "electrical response threshold" or equivalent effect whose variations may be examined under the same sets of conditions. But there is nothing in the electrical record that may be accepted a priori as the perfect analogue of the sensory threshold. We might specify a "detectable response," but this merely allows the criterion level to be determined by the exigencies of recording; any change in the "noise level" will alter the criterion. The custom has been, then, to specify some amplitude of response (usually a "small" one, in recognition of the concept of the threshold as a minimum value) which can be reproduced in a succession of circumstances so as to trace out the function. But a comparison of these functions with those obtained through psychophysical procedures rests on the assumption that the subjective threshold (regardless of the wavelength of the stimulus or the state of adaptation of the eye) reflects a constant or standard physiological response. But our "b-wave of standard amplitude" is by no means a "standard response." Responses of equal amplitude may vary in latency and in duration, indicating *differences* in underlying physiological processes. Further, the electrical response curves, in the typical ERG study, are drawn with reference to stimuli well above psychophysical thresholds in intensity and represent the eye at a different level of function. Such things must be borne in mind as we examine the data.

† Similar logic may be applied to the scotopic component of the negative a-wave (a_s-wave), which has a similar spectral sensitivity.[9]

the problem of disengaging it from other components. "Foveal stimulation" cannot eliminate scotopic responses because of the effects of scattered light, which I have detailed above. As Adrian pointed out, then, the photopic response is masked throughout most of the spectrum by the b_s-wave. While this masking is most pronounced in the dark-adapted eye, light adaptation seems not to abolish, but merely to reduce, the responses of the scotopic system. With higher levels of adaptation, those which may provide us with photopic responses in a relatively "pure" state, stimulus levels must be high and the responses are diminished in amplitude, making observation and measurement of responses difficult.

Furthermore, since Adrian first described the photopic component as "diphasic," we have regularly observed the positive b_p-wave only in association with its negative a_p-wave. There is not, as in the case of the scotopic components, a range of stimulus intensity below the level for the arousal of the negative component and in which isolated positive potentials are elicited.

Riggs, Berry, and Wayner[65] looked for photopic responses in the early minutes of dark adaptation, hoping in this way to find larger responses than those of the light-adapted eye. They remark the occurrence of "dual responses" at the red end of the spectrum but conclude from the sensitivity curve for b-waves recorded (Fig. 4*B*) that they are almost purely scotopic under these conditions.

Armington,[5,6] working with successively higher levels of light adaptation, obtained spectral sensitivity curves for the electrical

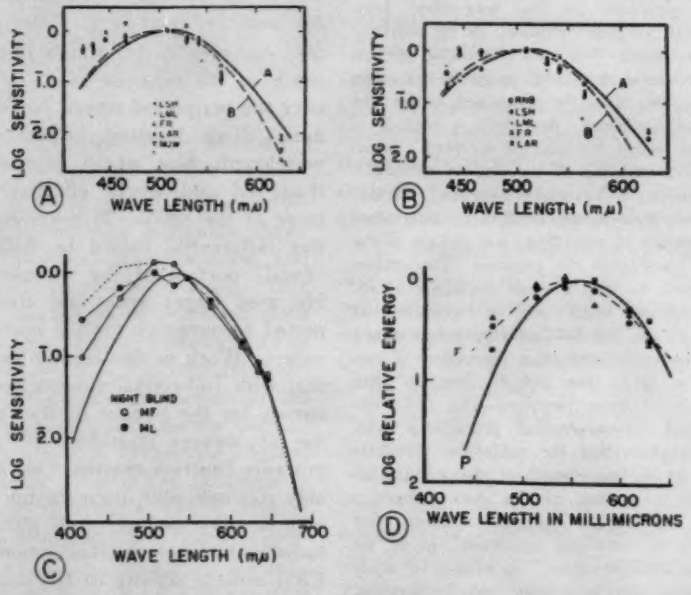


Fig. 4.—*A*, log of sensitivity as indicated by electrical responses of the dark-adapted eye. Data for five subjects plotted as points on the scotopic curve. (From Riggs, Berry, and Wayner.[65]) *B*, same for the light-adapted eye (after one minute of dark adaptation). Relative sensitivity essentially unchanged from that of dark-adapted eye. *C*, sensitivity of normal subjects (dotted line) and night-blind subjects (plotted points) determined by electrical-response measures with 7.1 foot-lamberts of light adaptation. Solid line is C. I. E. photopic luminosity curve. (Responses to single flashes.) (From Armington.[6]) *D*, a similar curve for electrical sensitivity of normal subjects with use of flickering light. Solid line is again the C. I. E. photopic luminosity curve. (From Johnson and Cornsweet.[62])

response ‡ which much more nearly resembled the photopic than the scotopic visibility curve, though elevated at the blue end of the spectrum (Fig. 4*C*). His best results were obtained with night-blind observers.[6] §

His results for the normal light-adapted eye have been duplicated by Johnson and Cornsweet,[49] who also went on to show that a very similar sensitivity curve could be obtained for responses to colored lights flickering at a rate of 30 per second (Fig. 4*D*). This was in line with a suggestion by Dodt [33] that the fluctuating electrical response produced by lights flickering at a rate above 20 per second results in the disappearance or coalescence of scotopic effects so as to leave a purely photopic remainder.

The elevation of all of these electrically determined photopic sensitivity curves toward the blue end of the spectrum, while superficially similar to what was seen earlier in the case of the scotopic curves, probably has a very different explanation. As Armington has pointed out,[7] most recent studies of the spectral sensitivity of the light-adapted eye [71,73] differ from those used to construct the standard C. I. E. curve in reporting greater sensitivity to the shorter wavelengths when areas outside the fovea are stimulated. The ERG photopic sensitivity curves are in good agreement with the newer psychophysical data.

The simplicity of the flicker technique has stimulated interest in the photopic ERG. Analysis of the response to flicker will undoubtedly provide insights into the nature of ERG components. As I am primarily concerned with single-flash work in this review, however, I shall not attempt to detail the work already done in this area.

But I should not conclude the discussion of the spectral sensitivity of the photopic ERG without raising the question of the possible multiple nature of the latter. In a series of papers Armington and his associates [8,10,15] have explored the effects of chromatic adaptation on the ERG. They conclude not only that rod mechanisms continue to function under photopic conditions but that specific color-sensitive mechanisms (one with a response peak to light of about 620 mμ being best invested with evidence) make contributions to the total recorded ERG. Possibly there are separate b_p-waves for each of several photopic color-response systems, each with slightly different response characteristics. The precise interpretation to be given these results is obscure at the present time. There is need for new conditions to be explored and for new techniques to be brought to bear on the photopic response.

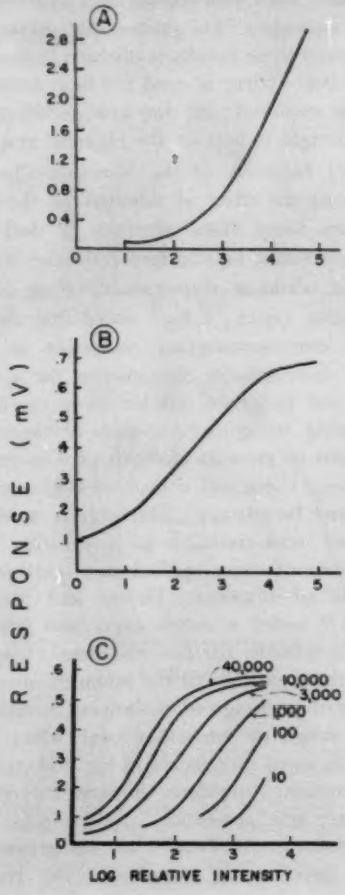
(2) *Intensity of the Stimulus.*—In considering the effect of intensity on the ERG I turn away from attempts to deal only with constant or standard responses to look for a while at responses differing as the stimulus varies. I have noted that the human electroretinogram comprises at least four discriminable components, the a_p-, a_s-, b_p-, and b_s-waves. Under those conditions favoring its appearance each of these may be seen to grow as strength of stimulus increases. Form and latency of response may likewise be affected. The large b_s-wave has proved most amenable to observation. The measure oftenest applied has been simple height of response. Dewar and McKendrick [31] noted a rough agreement between the amplitude of the response (b-wave) and the logarithm of the stimulus intensity. Modern work agrees in showing a considerable range of intensities over which successive equal increments in log I (logarithm of intensity) produce uniform increments in response amplitude. As in other approximations to Weber's law, the generalization fails at the extremes. De Haas [28]

‡ Armington follows Motokawa in speaking of the "x-wave." To determine amplitude he measures from the trough of the a- to the top of the b-wave.

§ Schubert and Bornschein [67] and Riggs [60] have also demonstrated that this component is to be found in the responses of the night-blind, with reduction of scotopic responses. Schubert and Bornschein [67] and Armington [6] have described its impairment in protanopes. Vukovich [70] remarked the absence of any "x-wave" and also the diminution of the a-wave in a totally color-blind subject.

observed responses of the frog eye with a d'Arsonval galvanometer, and in 1903 he published a curve relating response amplitude to stimulus intensity (Fig. 5*A*). Jolly [51] used stronger stimuli, and a plot of his data would show a levelling-off at the top. The entire function may be seen in Figure 5*B*, which I have taken from a paper by Hartline.[44] In 1937 Riggs [61] published data for the eye of the intact (curarized) frog not only for the condition of complete dark adaptation but also for various incomplete

Fig. 5.—Amplitude of the b-wave as a function of stimulus intensity. *A*, frog, data of De Haas, after figure in Kohlrausch.[54] *B*, frog eye, in situ, after Hartline.[44] *C*, frog eye, in situ, after Riggs.[61] Top curve is for complete dark adaptation (40,000 seconds); lower curves, for earlier stages in dark adaptation.



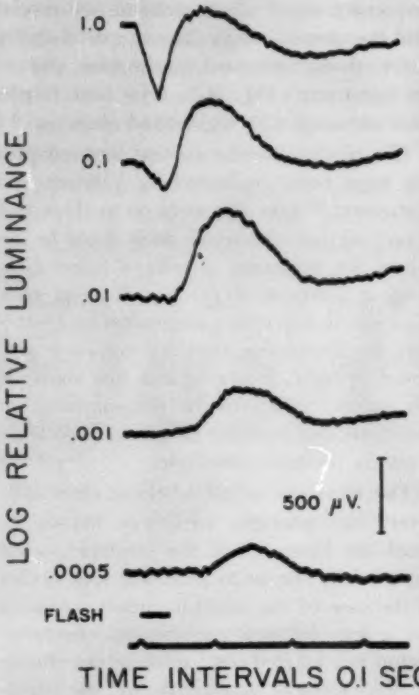


Fig. 6.—Tracings of oscillograph records of retinal responses of the dark-adapted human eye stimulated by 40-millisecond flashes of white light. The highest luminance used (log rel. lum. 1.0) is equivalent to about 1.5×10^3 foot-lamberts. At the higher levels both a- and b-waves appear. (From Riggs and Johnson.[60])

stages of adaptation. Some of his results are shown in Figure 5*C*.

Similar studies have been made of the responses of the human eye. But before one looks at the comparable graphs a few sample response tracings should be examined. One will see, then, some of the measurement problems that must be faced. Figure 6 represents a typical "intensity series" obtained by presenting 40-millisecond flashes of white light to the dark-adapted human eye. The positive component decreases in latency and increases in amplitude with increasing intensity. At the lower intensity levels, however, latency measurements are uncertain, because the response shows only a gradual departure from the base line, not an abrupt onset. For the higher intensities

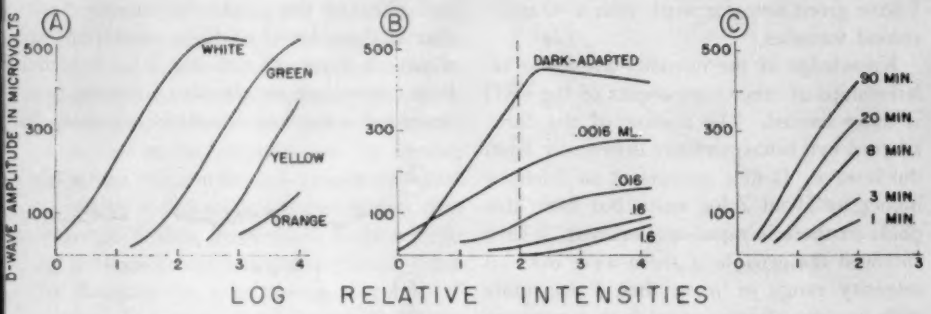


Fig. 7.—B-wave amplitude as a function of the stimulus intensity. *A*, lights of different wavelengths yield similar curves displaced on the intensity axis in accordance with their relative (scotopic) luminosity values except for the "orange" curve, where the difference in slope suggests intrusion of photopic responses in the records. All records are from the dark-adapted eye. (From Riggs, Berry, and Wayner.[66]) *B*, similar curves for stimulation with white light presented to the thoroughly dark-adapted eye and also to an eye maintained at each of four levels of constant light adaptation. (From Riggs and Johnson.[66]) *C*, stimulus light white. Eye dark-adapted for various lengths of time following a standard period of light adaptation. (Replotted from data in Johnson.[67])

it is the amplitude determinations that present the difficulty. The presence of the a-wave forces the investigator to choose an arbitrary base line. In most cases the b-wave has been measured as starting from the trough of the a-wave. This implied assumption that the negative process remains at a constant level during the time separating the peak of the a-wave from that of the b-wave affects determinations in the upper intensity range in the dark-adapted eye, where the b_s-wave is the chief positive component. With the light-adapted eye a-waves occur in every record.

Figure 7 shows the graphed results of three different studies of b-wave amplitude. In each of these a 40-millisecond flash was presented in a 7½ degree field. The left-hand curve in each graph shows the amplitude-intensity function for white light with the eye dark-adapted. The other curves indicate how the function is altered by changes in the wavelength of the stimulus, the light-adaptation level, and the time in darkness during dark-adaptation.

Which of the curves in Figure 7 represent relatively pure measures of the b_s-wave? Reflection indicates that the responses to white, green, and yellow stimuli, in Figure 7*A*, are relatively uncontaminated —up to their inflection-points; but the "orange" curve was drawn from dual responses which were partly b_p-wave. In Figure 7*B*, values to the left of the dotted line were taken from responses which were practically pure b_s-waves, but a-waves were present in the responses represented to the right of the line. The 8-, 20-, and 90-minute curves of Figure 7*C* are for the b_s-wave, but the 1- and 3-minute curves could be queried.

The upshot is that the b_s-amplitude of the dark-adapted eye appears rather simply related to log I, the relationship being approximately a straight line over a 1½ to 2 log unit range of intensities starting at about the level where the response is first clearly discernible above the base-line noise. We conceive the function as leveling off at both extremes but cannot rely very strongly on the quantitative measures outside the middle range. The slope of the curve in this middle region, and the maximum b-amplitude attained, will vary with the subject and with the degree of dark adaptation. Color of the stimulus has no effect if it is really the b_s-wave that is being measured. As we shall see later, the duration of the stimulus may affect the position of the curve on the intensity axis, but this does not detract from the generality of the results which

I have given here for work with a 40-millisecond stimulus.

Knowledge of the intensity-amplitude relationships of other components of the ERG is more limited. The a-wave of the dark-adapted eye behaves rather differently from the b-wave. It first appears at an intensity higher by about 2 log units, but from this point on there is rapid development. I have followed the growth of the a-wave over an intensity range of more than 2 log units with no sign of any approach to a response maximum. Indeed, using the slope of the a-wave as my measure,‖ I find that it is the logarithm of the response magnitude rather than the measure itself that falls more nearly along a straight line when plotted against log I. Amplitude-intensity functions published by Schubert and Bornschein [67] and by Burian [23] are in accord. I have attempted here no separation of a_s- and a_p-waves, both of which may be presumed to be involved. I might note that a hemeralopic subject of Schubert and Bornschein displayed a negative response greater than that of a normal subject, and the magnitude was linearly related to log I.

In work on the photopic components the positive and negative phases of the ERG have usually been treated together. The work of Armington and his associates provides the fullest picture of events. In these studies the "x-wave" amplitude is measured from the trough of the a_p-wave to the peak of the b_p-wave. With only moderate light adaptation, to a level of 1 foot-lambert, the responses clearly show the presence of the b_s-component except in response to red light.[5] With the eye adapted to levels of from 7 to 87 foot-lamberts, however,[6] the character of the responses is uniform for all parts of the spectrum. Increase in response amplitude appears proportional to the increase in log I. A few typical responses, and plots of the amplitude-intensity function at three levels of light adaptation, are shown in Figure 8 (*A* and *B*). A rather large increment in stimulus intensity produces but a small increment in response. In

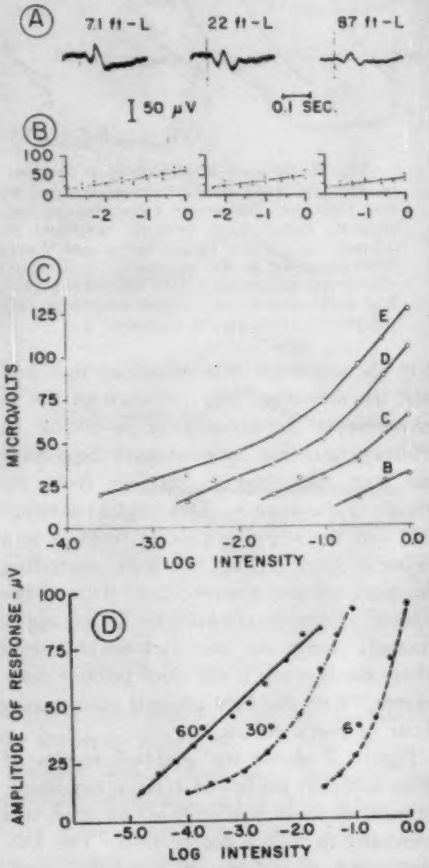


Fig. 8.—*A*, responses to a red stimulus (peak at 636μv.) presented to an eye adapted to three different levels of white light. (From Armington.[6]) *B*, plots of the amplitude of the ERG, in microvolts, as a function of stimulus intensity. Same color of stimulus and same three adaptation-levels as above. (From Armington.[6]) *C*, height of response as a function of stimulus intensity with stimulus areas subtending 2 degrees 8 minutes, 6 degrees 22 minutes, 15 degrees 50 minutes, and 26 degrees 4 minutes, respectively, beginning with the lower curve and going up. Red test flashes presented to eye light-adapted to about 2 foot-lamberts. (From Crampton and Armington.[28]) *D*, effect of stimulus diameter on the shape of intensity curves. Left-hand curve is for 60 degree stimulus area; other curves for 30 degrees and 6 degrees, respectively. Flickering red light; 10-millisecond flashes at 20 per second. (From Armington.[7])

‖ Because the latency of the b_s-wave decreases at high intensities, it must be supposed to cut into the course of the a-wave earlier, effectively "clipping" its peak and reducing the appropriateness of the a-wave amplitude as a measure of the negative potential.

later papers by Armington and Thiede [10] and by Crampton and Armington [26] the curves are shown with an upward turn, or acceleration, at the high-intensity end (Fig. 8*C*). In this work the test flashes subtended 30 degrees at the subject's eye. In another study,[7] in which photopic responses were elicited by a flickering stimulus rather than by single flashes, Armington finds a striking difference between the amplitude-intensity function for a large-area stimulus (60 degrees) and those for areas of 30 degrees or smaller (Fig. 8*D*). The intensity function for single flashes may also be affected by the area of the stimulus.

Turning next to temporal characteristics of the response, one notes that raising the intensity commonly speeds up the ERG. Gotch [37] demonstrated this for the frog eye in 1903. Figure 9 shows changes in latency of the b-wave of the dark-adapted eye in the cat and in man. The implicit time of the b_c-wave is affected similarly (cf. Monnier [56]), but the amount of the effect depends also on the duration of the stimulus. This follows from the protraction of the peak in the response to longer flashes at low intensities (see below). Latency is independent of flash duration.

Changes in the latencies of the photopic components are small and do not lend themselves to accurate measurement.

(3) *Duration of the Stimulus.*—Most published records of the human electroretinogram show it as primarily an "on-response" which, apart from certain pupillary artifacts, completely runs its course within the first 250 milliseconds following the onset of the stimulus. This seems to be true regardless of whether the duration of the latter is 5 or 500 milliseconds.

In discussing the effect of stimulus duration on the b-wave of the dark-adapted cat, Creed and Granit [27] make the following statement: "The duration of the stimulus is in our experience devoid of influence on the latent period. . . . The amount of potential developed is, however, generally diminished

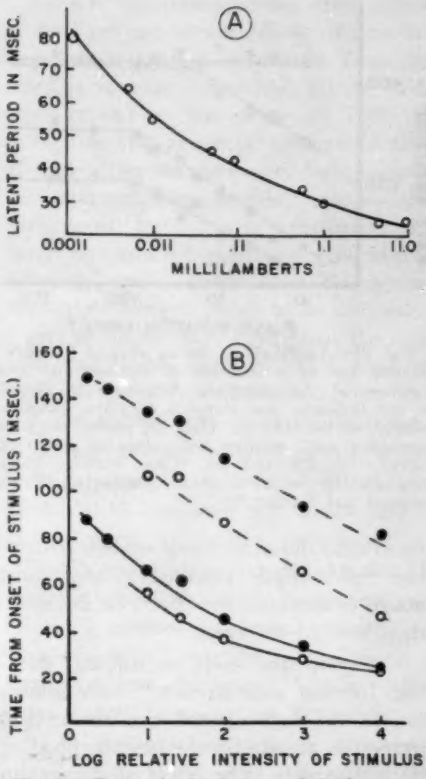


Fig. 9.—Change in the latency of the b-wave as a function of the intensity of the stimulus. *A*, from data obtained by Creed and Granit,[27] recording from the dark-adapted cat. *B*, data for man from Johnson and Bartlett.[48] Solid line, filled circles: dark-adapted eye. Solid line, open circles: eye adapted to a level of 2 foot-lamberts. Dotted lines show approximate functions for the implicit time (time to peak of response) at the same two levels.

by making the flashes shorter [though] . . . with the more intense stimuli this is not always so."

Müller-Limmroth [58] showed this to be generally true also for the human ERG. He used flashes varying from 5 to 1000 milliseconds and noted, with the stimulus intensity employed, a sharp falling off of the response when the duration of the flash was less than 20 milliseconds. Responses to all longer stimuli appeared practically identical. Most single-flash work on the human ERG has employed flashes of from 20 to 80 milliseconds. These were presumed to be above

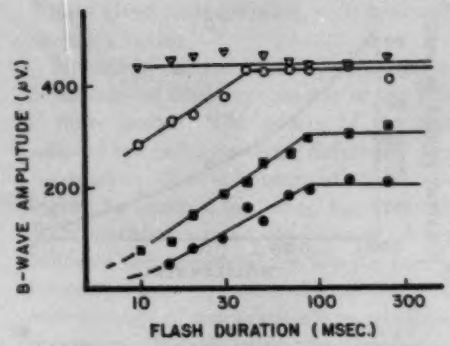


Fig. 10.—Amplitude of the b_s-wave of the dark-adapted eye as a function of the intensity and duration of the stimulus. Amplitude is plotted on the ordinate, and stimulus duration, logarithmically, on the abscissa. Data are plotted for four intensities with relative log values of 3.0 (top curve), 2.0, 1.0, and 0.5. Unit intensity value (log I=0.0) is about 2 foot-lamberts. (From Johnson and Bartlett.[48])

any critical duration (long enough to provide full-strength responses) and short enough to minimize the effects on the adaptation level of the eye.

In a systematic study of stimulus duration, Johnson and Bartlett [48] have shown that, in the dark-adapted eye, the critical duration is actually in the neighborhood of 100 milliseconds. The effect of changes in duration on the amplitude of the b_s-wave is shown in Figure 10. Examination of this figure shows a break in the curves at 100 milliseconds (the critical duration). Longer flashes may increase the duration of the response but not its amplitude. There is a maximum amplitude (about 430μv. in this instance) above which the response may not be driven. Once this has been reached, increases in the length of the stimulus flash—even though the "critical duration" of 100 milliseconds has not been reached—fail to elicit larger responses. Furthermore, they no longer appear able to affect the duration of the response. It seems rather as though this degree of stimulation "exhausts" some "reservoir" responsible for the b_s-wave.

Below the critical duration—and for responses below the maximum response amplitude—intensity and duration are pretty much interchangeable. The formula $It=k$ holds very closely. Records taken with the eye at a moderate level of light adaptation (0.2 foot-lambert) have yielded comparable results, with the important differences that the maximum response amplitude is reduced and the critical duration falls from 100 to around 30 milliseconds.

Previous work at this level of adaptation has shown that the responses have a scotopic spectral sensitivity curve; consequently, these changes probably indicate an altered scotopic response and not the involvement of a photopic system. In a recent study by Tansley,[68] however, it is reported that a very similar shift in critical duration, between the light-adapted and the dark-adapted state, occurs in the "pure cone" eye of the gray squirrel where it is associated, in both states, with photopic spectral sensitivity.

Alpern and Faris [4] and Wirth,[74] in studies of the effect of stimulus duration on responses of the dark-adapted eye, have included results for higher stimulus intensities than any employed by Johnson and Bartlett. Negative potentials are prominent in their records. Their data show that the amplitude of the undifferentiated a-wave increases with prolongation of the stimulus—at least up to stimulus durations of around 25 to 30 milliseconds.

I have already noted that the latency of the b-wave of the dark-adapted eye is not affected by duration. Implicit time, however, may be altered in that the prolonged responses evoked by low-intensity stimuli of longer duration (within the critical duration) appear to reach their maximum levels a little later than do responses to the briefer flashes. For this reason, the plotted functions in Figure 9*B* are referred to as "approximate," based as they are on an average measure for several different flash durations.

In a recent study, Bornschein, Goodman, and Gunkel [17] used a Grass photic stimulator with a 10-microsecond flash and found that with this brief stimulus implicit time approached a maximum value of about 125

milliseconds as the flashes diminished in intensity.

Data on a critical duration for the a_s-wave and for photopic components are not available. Any "critical time" for photopic responses would by practical necessity be much shorter than that for the dark-adapted scotopic response; thus, the employment of very brief stimulus flashes is one of the most successful devices for minimizing the b_s-wave in the ERG so as to view photopic components more effectively. The existence of off-effects points to a prolongation of response components throughout the course of stimulation in the light-adapted eye.

(4) *Locus and Area of Stimulation.*— In work on the human ERG neither locus nor area of stimulation can be controlled or adequately specified except through control of the angular extent and direction of the source external to the eye. To the extent that the eye distorts or diffuses, the area and locus of stimulation on the retina will be modified. If we should assume these processes to be negligible, and assume the ERG to arise within the light-stimulated portions of the retina, we might hope to study the differential sensitivity of various parts of the retina as the stimulus focused upon them. We would expect to find photopic components best aroused by central stimulation, scotopic responses at their best in rod-rich areas, etc. Increasing the area, within homogeneous parts of the retina, should add to the amplitude of the response without changing the time relations (latency, form). If, on the other hand, the eye scattered and diffused maximally all light that entered the pupil, neither changes in area nor changes in locus could effect changes in the ERG except as they altered the general level of illumination on the retina.

As one knows that the human eye provides a high degree of differentiation and resolution of seen objects, the extent to which the second of these two suppositions appears to govern production of electrical responses comes as a surprise.

Some of the contradictions which appear in the literature on the effects of area and locus of stimulation must arise from differences in conditions which appear to be more critical in this phase of ERG investigation than in others. Changes in angle of view affect the amount of light entering the pupil and must be taken into account. Differences in electrode placement may favor reception of potentials generated in different portions of the retina.[28] Light of shorter wavelengths tends to be diffused to a greater degree than light from the long-wave end of the spectrum and is thus less likely to reveal specific effects correlated with the precise locus of the retinal image.

Adrian,[2] looking for ways to differentiate between photopic and scotopic components of the ERG, compared records obtained by using central as against peripheral (annular) stimulus areas and reported that the photopic (fast, diphasic) component was relatively favored by central presentation. This has been confirmed by the work of Crampton and Armington.[26] The differences found, however, are but slight and tend to disappear as stimulus intensity is raised (Fig. 11*A*). And, whereas studies of the amplitude of the scotopic b_s-wave have sometimes reported an advantage in sensitivity for the periphery[19] or for the central region,[56] Crampton and Armington, using a 19 degree blue stimulus centered at various points out to 40 degrees from the fovea, emphasize the near equivalence of the responses when electrode placement and the amount of light entering the pupil at different angles of view are adequately controlled or corrected for.

A most compelling argument for the potency of stray or scattered light has been the evidence offered by Boynton and Riggs[19] and by Asher[11] showing that light directed onto the blind spot is as effective in eliciting the b_s-wave as though it were falling on those neighboring regions of the retina where visual sensitivity is acknowledged.

Boynton [18] and, more recently, Crampton and Armington [26] have advanced an interpretation which emphasizes that the retinal response to stimuli of circumscribed area is comprised of two components, one from the intensely stimulated focal area and the other from the suffused nonfocal regions. The importance of the nonfocal component was emphasized by Boynton and Riggs when, using areas with visual diameter varying from 1.25 to 12 degrees, they reported that (b_a-wave) responses of a given magnitude had the same latency and were indistinguishable from one another in shape regardless of the intensity-area combinations used to produce them. With larger areas, however, changes in form appeared.[18] The influence of the focal component may also be traced in a tendency for the intensity curves obtained by Crampton and Armington with small stimulus areas to start leveling off at a lower response amplitude than when larger areas are involved. The latter curves, and the derived curves showing intensity of stimulation necessary to elicit b_a-waves of specific magnitudes with various stimulus patch areas, are presented in Figure 11*B* and *C*. The straight-line plots in Figure 11*C* bear out Boynton and Riggs' earlier statement that the relationship between area and intensity, for this function, is expressible by the formula $IA^k=C$. The value of k is near to unity, a value that would indicate interchangeability of area and intensity.

The difficulty of controlling or defining the retinal locus of the stimulus makes such area or locus studies of the human ERG rather poor tools for investigation of the function of specific retinal areas.

(5) *State of Adaptation.*—Under this head I shall treat the changes in response which occur during the course of dark adaptation and the characteristics of responses recorded at various levels of light adaptation.

Since the time of Kühne and Steiner [55] it has been known that the responses of the light-adapted eye are smaller. Einthoven

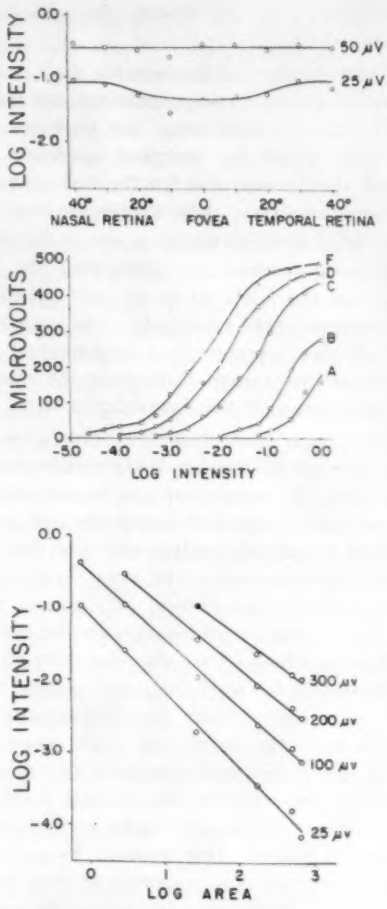


Fig. 11 (from Crampton and Armington [26]).—*A*, intensity of stimulation necessary to elicit (photopic) responses of fixed magnitudes (50μv. responses, upper curve; 25μv. responses, lower curve) at selected retinal positions extending to 40 degrees on either side of the fovea. Red test flashes were used. Sensitivity is greater near the fovea when the response criterion is small. Stimulus patches were 19 degrees in diameter. *B*, height of the b_a-wave as a function of stimulus intensity for various areas. Blue test-flashes presented to the dark-adapted eye. Diameter of the stimulus patches for each curve (from top down): 30 degrees, 15 degrees 50 minutes, 6 degrees 22 minutes, 2 degrees 8 minutes, 1 degree 4 minutes. (Note: A similar set of curves for the photopic response appears in Figure 8*C*.) *C*, these curves are derived from those of Figure 11*B*, above. They represent the intensity of stimulation required to elicit b_a-waves of specific magnitudes. (Top curve is for 300μv.; others, for 200μv., 100μv., and 25μv., respectively.) Log area 2.82 corresponds to the 30 degree stimulus patch.

and Jolly [35] noted that early in dark adaptation the frog's a-wave might be elicited with very little of the positive b-response, while later in dark adaptation a relatively weak light produced a large b-wave but no a-wave.

Adrian [2] reported a similar effect in the human eye. His diphasic "photopic response" was relatively less prominent, and the slower b-wave was more prominent, in the dark-adapted eye. There have been several studies of the growth of the human b-wave while the eye dark-adapts. In the first of these, Karpe and Tansley [53] observed how the response to a flash of constant intensity gradually increases in amplitude. They were impressed by the similarity of its growth to concurrent changes in the subjectively determined light threshold.

Johnson,[47] Johnson and Riggs,[50] and Best [13] employed the technique of the "standard response." ¶ A response criterion was set: a b-wave of small, but standard, amplitude. Whereas a strong flash was required early in the dark-adaptation session, toward the end a much weaker one sufficed to evoke the response. Johnson and Riggs noted the compound nature of the b-wave and attempted to study the b_s-wave in comparative isolation. For this purpose they used a low level of preadaptation light (1.73 foot-lamberts), and flashes were low enough in intensity so that neither a-waves nor b_p-waves were characteristically elicited. Neither their electrical response nor psychophysical dark-adaptation curves, obtained under similar conditions for purposes of comparison, showed any rod-cone "break" (Fig. 12*B*). It was noted that the precise shape of the electrical response curve depended on the response amplitude taken as a criterion. Despite obvious dissimilarities between the electrical and the psychophysically determined curves, the authors concluded that adaptation of the scotopic system underlay both. Best's study led to similar conclusions. But in his work [13,14] and in that of several other investigators [3,12,16] higher levels of preadaptation and, to excite responses in the early stages, stronger stimulus flashes were used. Under these circumstances, photopic components are elicited. With a judicious arrangement of stimulus factors, electrical dark-adaptation curves may be obtained in which a "break" occurs, analogous to the well-known rod-cone break (Fig. 12*C*). Such curves are based on measurements of a duplex b-wave in which the b_p-wave component is more prominent in the early minutes of dark adaptation, the b_s-component becoming more prominent later. The difficulty of separating b_p from b_s under these circumstances and the consequent arbitrariness of measurement have been demonstrated and discussed by Auerbach and Burian.[12] But the relationship of the early developing b_p-wave and the later appearing b_s-wave to photopic and scotopic dark-adaptation processes, respectively, seems firmly substantiated.

Examination of the a-wave during the course of dark adaptation has shown a similar division into earlier- and later-developing components (a_p and a_s), with the growth of the a_s-component closely paralleling that of the b_s-wave, though requiring higher stimulus intensities for its elicitation [9,12,13,16] (Fig. 12*D*). As with the b-wave, proper selection of conditions and response criteria may show a "rod-cone break" in the dark-adaptation curve.[16]

As may be seen by examining data for early phases of dark adaptation in the studies just cited, a_p- and b_p-waves also show a limited growth in the dark.

During dark adaptation latency and implicit time of the b-wave are reported to lengthen.[13,47] This presumably represents a real slowing and not merely a shift of observation from b_p- to b_s-wave. The relationships of such changes to stimulus duration, area, etc., have not been worked out.

For changes in the response when the eyes are accustomed to various steady levels of adapting field luminance we may refer primarily to two papers: that of Riggs and

¶ See discussion of the rationale of the "standard response" technique in footnote on p. 571.

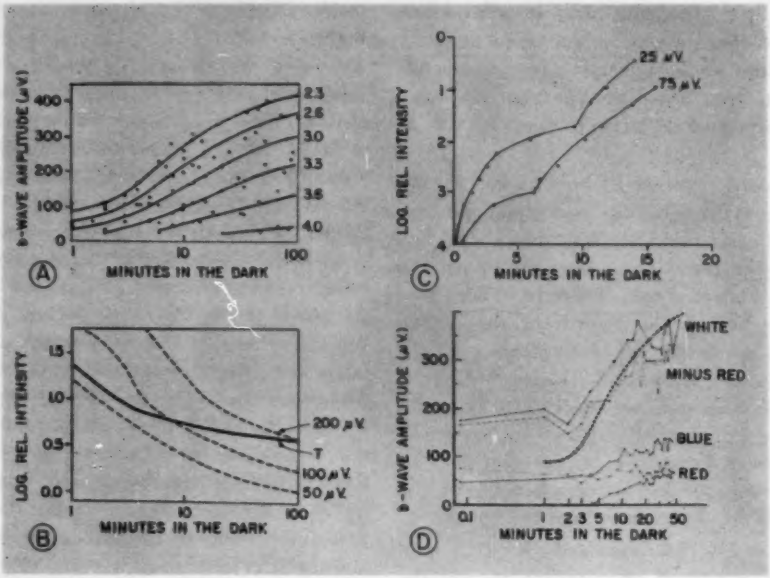


Fig. 12.—Dark adaptation as reflected in the ERG. *A*, magnitude of response (b_s-wave) on stimulation by white light during dark adaptation. Numbers on curves show log density of neutral filters used. Full luminance (log density 0.0) was about 1,500,000 foot-lamberts. (From Johnson and Riggs.[66]) *B* (also from Johnson and Riggs [66]), dotted curves are derived from those of Figure 12*A* and show relative luminance required to produce responses of 50μv., 100μv., or 200μv. after various periods of darkness. A psychophysically determined dark-adaptation curve, *T*, was obtained under similar conditions of preadaptation. This curve, actually obtained with much weaker stimuli, has been shifted along the intensity scale in plotting. *C*, curves from Best and Bohnen [34] similar to those in Figure 12*B* but with a "rod-cone break." The preadapting light level was higher in this study, however, and recorded responses are not differentiated as b_p- or b_s-waves. *D*, plot similar to Figure 12*A* but showing growth of a-wave. Color of stimulus is indicated for each curve. "Red" curve shows no evidence of development. Bottom curve (unlabeled) is an attempt at measurement of a_s-component in the "blue" growth independently of the a_p-component. Dotted line shows b-wave growth for comparison. (From work of Armington, Johnson, and Riggs.[6])

Johnson [66] for low luminance (about 1.6 foot-lamberts and below) and that of Armington [6] for levels from 7.1 to 87 foot-lamberts. In the completely dark-adapted eye scotopic responses predominate, though photopic responses occur also and achieve considerable prominence at the higher stimulus intensities. At the upper end of the range it is the scotopic portions of the response which are nearly, if not completely, eliminated. The changes, both in the form of the response and in its amplitude, are clearly observable in Figure 13 and will not be detailed here. It is obvious that scotopic components may be greatly diminished by the presence of even a weak adapting field and that all components are progressively reduced by successively higher levels of adaptation. But, as light adaptation also alters the critical duration, at least that of the b_s-response,[48] it is clear that effectiveness of an adapting light in reducing this component must depend in part on the duration of the test flash employed to evoke it. The 40-millisecond flash used to obtain the records in Figure 13 is well below the 100-millisecond critical duration established for the dark-adapted eye but is probably above the critical duration for all components at the more highly light-adapted levels. The picture would be altered considerably by the use of longer or shorter stimulus flashes.

Once light adaptation has been raised to a level at which only photopic responses are

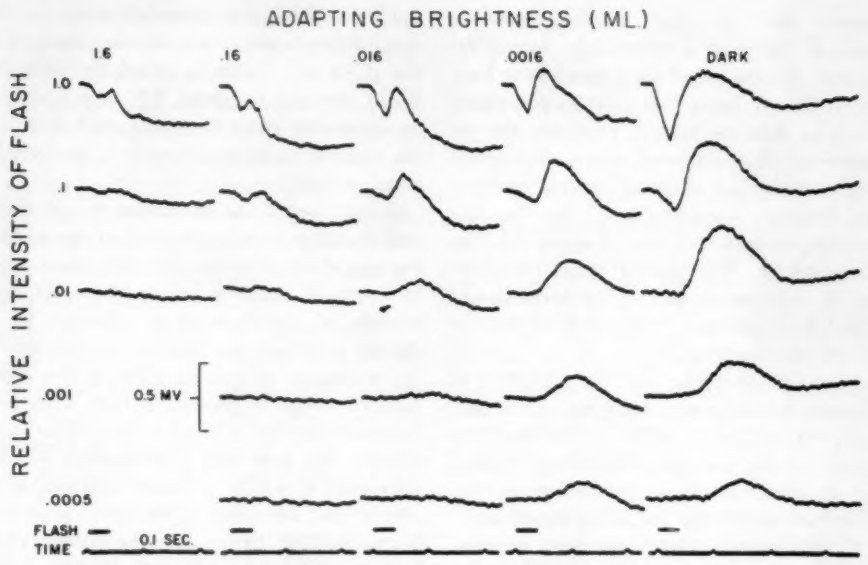


Fig. 13.—Human retinal responses at each of five levels of adaptation. Each record shows the response to a 40-millisecond flash of white light. Log relative intensity of flash is indicated at the left, with the highest level (1.0) corresponding to a luminance of approximately 1,500,000 foot-lamberts. (From work of Riggs and Johnson.[68])

observable, it is reported[6] that responses merely grow smaller at higher levels of adaptation, with no remarked change in general contour.

Analysis of the B_s-Wave

The b_s-wave is by now so familiar to all who work with the human electroretinogram that it is generally considered incapable of providing any surprises. Most thought is currently given to methods of circumventing its appearance (as by using flicker) in order to examine other components. Before setting it completely aside it might be well to summarize impressions of its simple nature.

Work with colored stimuli has persuaded us that the process underlying the b_s-wave is scotopic. Its "sensitivity curve" is essentially the rod luminosity (visibility) curve. Differences in the wavelength of the stimulus are considered ineffective except as they also represent differences in scotopic luminosity. Such differences may be fully compensated for by changes in intensity. The growth of the reponse during dark adaptation provides additional confirmation of its scotopic character.

"Locus of stimulation," which might be expected to provide further evidence, particularly through an analysis of differences in the size of the response to "central" as compared with "peripheral" stimulation, proves not to be a very effective factor when one is working with the b_s-wave, because the response to any light, however circumscribed the source and regardless of where on the retina its focal image may lie, is largely a response of the whole retina to light scattered over it. This fact imposes severe restrictions on the interpretation of much of the work that has been done on the scotopic b-wave. Since it is well recognized that temporal aspects of the response (e. g., latency, implicit time) are affected by intensity, it is clear that an inhomogeneous stimulus, one that effectively consists of an intense "focal" area surrounded by a large dimly illuminated yet responsive "nonfocal" area, should be expected to produce a re-

sponse that is also inhomogeneous—a "slurred" or even a discernibly duplex response. A very *small* focal area has at least the virtue of being too tiny to contribute much, so that one records primarily the response of the peripheral areas. Somewhat larger stimuli are likely to provide interesting b-wave "complications," to the excitement, confusion, or disgust of the experimenter. The ideal stimulus for eliciting the response in its simplest form should afford homogeneous illumination of the entire retina (Boynton [18]).

Turning attention to the effects of stimulus *intensity* and *duration* on the size and form of the b_s-wave, I shall heed the dictates of the paragraph above by employing, as illustrations, records taken in this laboratory under the following conditions.

1. Full-retinal stimulation was achieved by directing the subject's eye into an integrating sphere which was illuminated by the flashes.

2. All stimuli were presented through a minus-red filter (Wratten 44A) to minimize photopic involvement. (I should have used blue or violet light had I been able to obtain sufficient luminance.)

3. One full hour of dark adaptation immediately preceded each recording session.

4. Stimuli were presented no oftener than one per minute.

In each recording session I tried to record responses to a series of intensities ranging upward, in 0.3 or 0.4 log unit steps, from one that gave a minimal response to one having a maximum luminance of about ½ foot-lamberts and for each of six different stimulus duration values: 10, 20, 40, 80, 160, and 320 milliseconds. All records are from the same eye, and all records presented in juxtaposition were recorded on a single day and in continuous session. Three such sessions were run. In the third the eye was atropinized to reduce the rather irregular pupil artifact that interfered with the later portions of the responses. For this one session, then, I had a controlled pupil—provision for which should certainly be made in any more extensive study of the quantitative aspects of the response. On the third day I also extended the intensity series upward to about 2.5 foot-lamberts. In discussing these records I shall draw on the results, summarized earlier, for "small-area" stimuli.

I may begin the discussion of *intensity* and *duration* as stimulus factors by repeating and illustrating the fact that, while there is a critical duration below which the amplitude of the b_s-wave is affected by a change in either, any attempt to compensate for a change in one by altering the other fails of complete success in that, while the response may be restored to its original amplitude, the new time relationships will be different; it will be a faster response, or a slower one, according as the restitution was brought about by raising the intensity or increasing the duration of the stimulus. This is illustrated in Figure 14*A*, where responses of approximately equal amplitude (about 175μv.) elicited by 10-millisecond and 80-millisecond flashes are presented. A difference of about 15 milliseconds in latency and 30 milliseconds in implicit time appears. The point is even more strikingly made in Figures 14*B* and *C*, where the results of altering stimulus intensity or duration may be compared. (Note that the response to the 20-millisecond 0.05 foot-lambert stimulus appears in both series.)

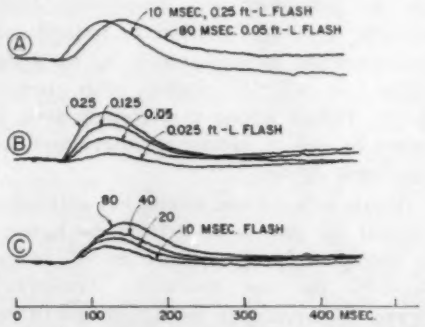


Fig. 14.—*A*, responses to 10- and 80-millisecond flashes with stimulus intensity selected to give the same amplitude of response (about 175μv.). *B*, responses to flashes of varying intensity but of constant duration. *C*, responses to flashes of different durations but of constant intensity.

To start the elucidation I shall begin by making two simple assumptions which may be modified later as required.

1. *Consider that one may identify a basic "unit" of b_u-response elicited by a brief flash of light of low intensity.* To be concrete, take a 10-millisecond stimulus yielding, at "unit" intensity, an easily measurable (a comfortably perceptible) response with an amplitude of perhaps 30μv. Such responses may be observed. One is drawn, as true-to-life as possible, in Figure 15*A*. It is shown with a latency of 90 milliseconds, with a peak at 140 milliseconds. It returns to the base line 210 milliseconds after the beginning of the stimulus.#

2. *Assume that the eye's ability to respond remains unaffected by the presentation of this "unit" stimulus and by production of this "unit" response,* so that successive 10-millisecond segments of the stimulus elicit identical units of response which successively summate.

The "unit response" to a 10-millisecond stimulus of unit intensity is presumed, throughout this paper, to have the following form:

Msec. After Beginning of Stimulus	Amplitude of Response, μv.	Msec. After Beginning of Stimulus	Amplitude of Response, μv.
10–90	0	160	23
100	8	170	18
110	15	180	13
120	21	190	8
130	27	200	4
140	29	210–	0
150	27		

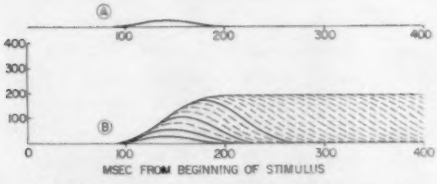


Fig. 15.—*A*, illustrative "unit response" to a 10-millisecond stimulus of low intensity. *B*, response curves generated by assuming that each additional 10 milliseconds of stimulation contribute an additional "unit response," with identical amplitude and time characteristics. The 10-, 20-, 40-, and 80-millisecond response curves are emphasized for comparison with responses in Figure 14*C*. With the "unit" we have assumed, the maximum response amplitude is about 200μv.

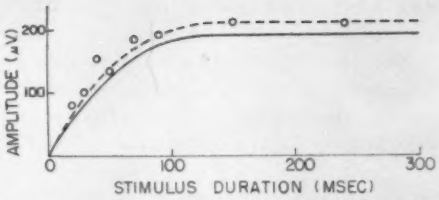


Fig. 16.—Amplitude of the b_u-wave as a function of stimulus duration. Points are replotted from experimental data shown in Figure 10 (bottom curve). The solid line drawn here was constructed by taking b-maximum values from each of the individual curves of Figure 15*B*, (the summation of "unit responses"). The slight displacement of the points above the line may be considered as reflecting a small difference in the luminances of the actual and the hypothetical stimuli. The difference is thus of no consequence in our argument. A slight change in scale, to allow for this difference, produces the dashed curve.

By graphic integration one may now compute the responses that would follow upon stimulation by flashes of various lengths. These are depicted in Figure 15*B*. The 10-, 20-, 40-, and 80-millisecond resultants are emphasized in the drawing for comparison with the actual response tracings in Figure 14*C*. The agreement is good. One observes that, whereas latency remains constant, implicit time should increase from 140 milliseconds for the 10-millisecond stimulus to about 145, 158, and 182 milliseconds for the 20-, 40-, and 80-millisecond stimuli, respectively. Examination of our actual records shows average implicit times of 140, 140, 153, and 175 milliseconds for the corresponding responses.

It is also apparent that a "critical duration" must appear at about 100 milliseconds, inasmuch as the "unit response" is of about that duration. This means that the response occasioned by later portions of a longer stimulus occurs too late to summate with the early part of the response and so serves merely to maintain rather than to elevate the b-wave. This accords with the facts. From the set of artificial curves one may also plot a graph showing amplitude of the b_u-wave as a function of stimulus duration. Figure 16 shows such a curve drawn through a set of experimental points taken from the

data of Johnson and Bartlett,[48] obtained without full-retinal stimulation.

That there is a limit to the application of my assumptions is seen, however, when I observe the implication that responses to long flashes should rise not to a peak but to a "plateau," returning to the base line only after the cessation of the stimulus. But even this does hold, approximately, for low-intensity stimuli, though not when stimulus levels are raised. As one ordinarily observes the electroretinogram, using stimuli which excite maximum or near-maximum b-waves, one seems to be dealing with a pure "on-effect," but such is not the case. The production of a response apparently depletes some sort of response reservoir. The higher the level of the response elicited, the more rapid and severe is the depletion. As one approaches an intensity level 2 log units above the response threshold one finds that this "depletion" reaches a stage where even a brief flash seems to exhaust the entire reservoir. The result is a maximum b-wave and the diminished critical duration which, as was noted earlier, characterizes the higher intensity levels. The response tracings in Figure 17 illustrate these points. At the weakest intensity level even the response to the 320-millisecond flash maintains its amplitude with little evidence of depletion. At the middle light level depletion is noticeable. At the highest level, where one just begins to see evidence of an

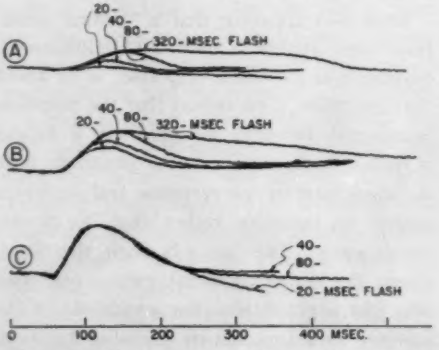


Fig. 17.—Response tracings at three intensity levels. *A*:0.0125 ft.-L. *B*:0.05 ft.-L. *C*:0.25 ft.-L. Stimulus duration is indicated for each tracing.

a-wave, the available "reservoir" seems to be entirely consumed even by the effects of a 20-millisecond stimulus and no further response may be observed to continued stimulation.

I find it necessary, then, to modify the second of my assumptions. The eye's ability to respond electrically is affected during the course of the response, an effect which we have spoken of metaphorically as "depletion" of a "reservoir." A proper quantitative study of this "depletion," which may well be related to the diminution of response observed as the eye becomes light-adapted, must await work with an eye in which pupil-accomodatory artifacts may be controlled. It may well be that animal subjects would be more appropriate than human subjects for this sort of research.

Turning from the factor of *duration* to that of *intensity* of the stimulus, I may again try making one or two simple assumptions.

3. *Assume that the amplitude of response increases or decreases in proportion to the change in the logarithm of the stimulus intensity.* I shall assume that all parts of the "unit response" are uniformly affected. Making this specific, I shall add 20μv. to the unit's maximum amplitude for each 0.1 log unit increase in intensity *until a ceiling of 400μv. is reached.* This rate of growth and ceiling are fairly typical.

4. Accepting the original "unit stimulus" latency of 90 milliseconds when log I is 0.0 (the original base intensity), *assume that changes in the logarithm of the stimulus intensity result in inverse changes in log latency.* Taking my inspiration from empirical observation, I shall suppose the change in log latency equal to one-sixth of the change in log intensity. The resulting relationship may be expressed in the following formula:

$$\log \text{lat.}_s = 1.9542 - \log I/6$$

The value 1.9542 is, of course, the logarithm of 90, the latency, in milliseconds, at the intensity level which I chose previously as a base.

To test whether the addition of these two assumptions provides any reasonable picture

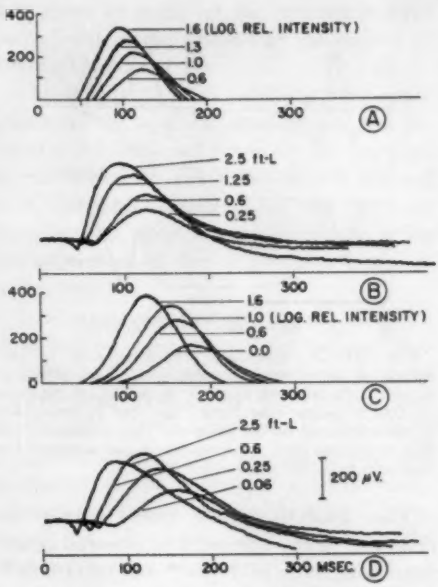


Fig. 18.—*A*, curves constructed from the "unit response." These curves are for a 10-millisecond flash. Amplitudes and latencies are determined according to my Assumptions 3 and 4. The 0.0 intensity level would represent the basic "unit response." *B*, actual response tracings for 10-millisecond flashes of a similarly graded intensity series. Calibration given in *D*, below. *C*, curves as in *A*, but calculated for 80-millisecond flashes. *D*, comparable series of tracings of responses to 80-millisecond flashes.

of the behavior of the b-wave I have employed them to generate the series of response curves shown in Figures 18*A* and *C*, for 10-millisecond and 80-millisecond flashes. To obtain these curves the "unit response" is first summated or integrated over the appropriate duration and then multiplied by a factor which raises its peak amplitude to the level indicated by Assumption 3; finally it is moved bodily along the time axis in conformity with the statement about latency embodied in Assumption 4.

In Figure 18*B* and *D*, are presented tracings of actual responses taken in the comparable circumstances. Though there are obvious dissimilarities between the computationally derived curves and the electroretinograms, there is also a hopeful degree of correspondence. One is not too far from a set of principles providing a rather accurate descriptive account of the b-wave. Matters might be improved somewhat by positing a slightly different "unit response" or perhaps by using as an alternate to Assumption 4 a supposition that the entire time course of the response, not merely its latency, is altered by changing the stimulus intensity. But it is not profitable to press toward such refinements so long as the quantitative aspects of the "depletion" effect, which I have mentioned previously, and of the concurrent a-wave remain vague.

Several methods of studying the depletion effect suggest themselves. One might observe the decline directly in responses to long flashes (cf. response to 320-millisecond flash in Fig. 17*B*). If one puts sufficient faith in the hypothesizing of the last few paragraphs one might compare the heights of observed and computed b-waves, taking the difference to represent depletion. Another technique might be that of taking records in response to stimuli of progressively longer duration—e. g., every 10 milliseconds, in order to see what is added to the response by each new 10-millisecond increment. In such a study artifacts would have to be controlled rather well. One would also need to acquire a knowledge of the accompanying negative (a-wave) process.

One study related to this "depletion," and pointing up its relation to light adaptation, has been made by Dodt.[34] He has worked on the recovery phase following cessation of a 500-millisecond stimulus at an intensity level high enough to show only the slightest of off-effects (i. e., subsidence of residual b-wave at "off"). Flashes presented immediately after the cessation of the first stimulus yield but a meager response. About a second in darkness is required before a second flash if it is to excite a response of normal vigor. Dodt refers to this depression of response as an illustration of postexcitatory inhibition. In the metaphor of the present paper I would speak of the time required "to refill the b_s-process reservoir."

There is little more to add to this discussion. This has been an historical and descriptive summary relating primarily to

the b_s-wave. It is hoped that the concept of the "unit response"—a response conceived as a function with both temporal and amplitudinal dimensions, but referred back to a specific small time segment of the stimulus—may prove helpful in an approach to other components of the ERG. Comparison of the responses of the light- adapted eye to a brief (10-millisecond) and to a longer (500-millisecond) flash of high intensity (Fig. 19) suggests the possibility of this approach. If one tries to resolve the 10-millisecond response into "unit" b_p- and a_p-responses, as in Figure 20*B*, and if one

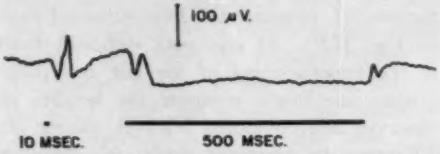


Fig. 19.—Tracings of the response of the light-adapted eye to a 10-millisecond and to an immediately following 500-millisecond stimulus. White light of about 1,000,000 foot-lamberts equivalent luminance was presented here in Maxwellian view with a 12 degree stimulus diameter.

summates these successively so as to represent 50 such responses at 10-millisecond intervals (i. e., "steady stimulus" lasting 500 milliseconds), the resultant, as shown in Figure 20*C*, is remarkably like the actual response to a 500-millisecond flash. The separate a_p- and b_p-components are shown in Figure 21. It may be supposed that either depletion or a slight change in the tempo of the individual unit-components accounts for the fact that the off-effect, which should otherwise be a perfect inversion of the on-response, is actually a bit smaller.

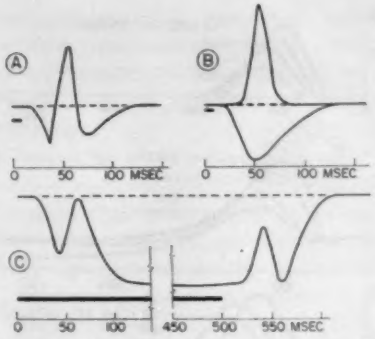


Fig. 20.—*A*, schematic representation of response to an intense 10-millisecond flash delivered to the light-adapted eye. *B*, hypothetical analysis of this response into "unit" a_p- and b_p-responses. *C*, the resultant of 50 pairs of "unit responses" of this sort combined successively in simulation of a response to a 500-millisecond flash.

This analysis, apart from making no reference to any c-wave, is in essential agreement with those of Piper [60] and of Granit [39] (Fig. 2*C* and *D*). Indeed, it resolves the point of difference between them by providing, actually, two "d-waves," that of Piper preceding and that of Granit following the decline of the positive process. Unfortunately, data are not sufficient for a discussion of experimental parameters here, and much research must be done before this isolated "analysis" may be incorporated into a full and coherent account of the behavior of the photopic components of the electroretinogram.

Summary

This is a history and summary of investigations leading toward analysis of the human electroretinogram, with special atten-

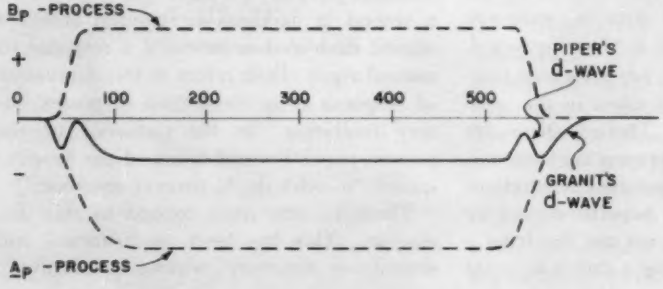


Fig. 21.—Resultant of 50 "unit responses," both b_p- and a_p-components indicated, illustrating the origin of the several "waves" in the photopic ERG. Time scale in milliseconds.

tion given to work on the scotopic b-wave carried out in the psychology laboratory of Brown University.

Changes in the ERG resulting from alterations of the spectral composition, the intensity, the area and locus, or the duration of the stimulus—and differences correlated with the adaptation-level of the eye—are described in some detail. Emphasis is on the expression of these changes in quantitative terms.

It is suggested that the human ERG be described by reference to four "waves," two of which, the photopic and scotopic b-waves (abbreviated as b_p- and b_s-waves), represent postivity at the cornea and two of which, the photopic and scotopic a-waves (a_p- and a_s-waves), represent negativity. It is recommended that the designation "x-wave," introduced by Motokawa and Mita for the photopic b-wave, be dropped.

An analysis of those changes in the form of the b_s-wave of the dark-adapted eye that accompany variations in the duration and intensity of the stimulus is presented. This presentation is aided by reference to a hypothesized "unit response" having a duration of about 100 milliseconds and occurring in response to a 10-millisecond segment of the stimulus. Derivations from this hypothesis accord well with observations on the change in implicit time as stimulus duration is lengthened and also provide a basis for the appearance of a "critical duration" in work with low-intensity stimulation.

The existence of a maximal response, the shortening of the critical duration at higher intensity levels, and the failure of the b_s-response to maintain amplitude during prolonged stimulation by light of moderate of high intensity are explained by invoking a "depletion" effect, which is compared to the draining of a reservoir.

The unit-response technique is tentatively extended in interpretation of responses of the light-adapted eye, where two processes, one positive and one negative, seem to produce the initial a_p- and b_p-waves and also, at "off," a d-wave complex that is a mirror-reversal of the on-effect.

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The Electrical Response of the Human Eye in Far-to-Near Accommodation

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I. Introduction

Schubert[1] has recently shown that the accommodation of the normal human eye from far to near is accompanied by a direct-current change in potential which may be recorded from suitable electrodes placed on the conjunctiva in the region of the limbus. Schubert used the shell of a contact lens, from which the corneal section had been removed, onto which a silver-silver chloride electrode was attached. With suitable anesthesia of the cornea, this technique is probably satisfactory for making a few measurements. However, when a large number of measurements are required, over extended periods of time, we have found that severe damage to the corneal surface can result because the cornea of the eye rubs against the border of the lens, from which the corneal section was removed, whenever the eye chances to look to one side or the other. The present paper will describe a safer technique for recording these potentials which consequently permits more extended measurements over longer periods.

It will also describe a number of experiments which have been carried out in order to establish the possible source of the recorded potential. Some preliminary results will be described which show how the magnitude of the voltage recorded varies with the separation of the far and near targets and with the magnitude of the change in the accommodation responses of the eyes.

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Department of Ophthalmology and Vision Research Laboratories, The University of Michigan.

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II. Method and Procedure

An impression is made of the subject's eye, and a standard-fluid contact lens is manufactured from the resulting mold and ground to render the eye emmetropic. The lens is then filled with buffer solution to which a drop of fluorescein dye is added, inserted on the subject's eye, and examined for the extent of the fluid pool of the lens. A small mark is made on the scleral section of the lens, several millimeters outside the region of this pool on the temporal side at 3 o'clock. The lens is removed, and a 2 mm. circular hole is drilled through the lens at this point. A methacrylate (Plexiglas) tube (11 mm. long, of 2 mm. outside diameter and 1 mm. inside diameter) is fitted into the hole, and the joint is sealed with fluid methyl methacrylate, which is allowed to dry for about an hour. A wire lead is inserted into the tube and sealed in position by packing the remainder of the tube with fluid indium which is just above its melting point. (A small syringe and 23-gauge hypodermic needle may facilitate this operation.) After the indium has cooled, it is buffed until smooth. A thin coat of silver print is then painted so that it makes contact with the indium and with the region of the sclera over the ciliary muscle. Contact between the electrode and the liquid pool of the lens should be avoided. The effect of the pattern of the electrode upon the adequacy of the electrode has not been systematically investigated. We have obtained satisfactory results from a roughly square surface 9 mm. on each side, extending equally far above and below the indium solder and posteriorly to the edge of the lens, but no farther anterior than the an-

terior edge of the indium joint. The silver print is allowed to dry, and its inside surface is buffed smooth. The electrode is chloridized in the usual way and is then ready for use. As the indifferent electrode, a chloridized Grass EEG electrode is attached with some electrode jelly to the bone behind the left ear. In order to minimize D. C. drift, when not in use the two electrodes are connected together and placed in physiological saline solution.

The essential features of the experimental arrangement were the same as those previously described.[2] The subject sat in an electrically shielded but well-illuminated room and fixated the mirror image of a vertical plumb line located 192 cm. from his spectacle plane. An aluminum ring 11.5 cm. in diameter could be localized at any desired near distance. A single strand of black surgical thread was stretched tightly across the horizontal diameter of this ring. The subject observed a circular white background about 2.5 degrees in diameter and having a luminance of about 3.2 foot-lamberts superimposed upon which were located a far (vertical) and a near (horizontal) black line which intersected in the center of the field much like the cross hairs of a reticle. The subject's task was to maintain fixation on the point of intersection of the horizontal and vertical lines but (on command) to bring into focus either the horizontal (near) or the vertical (far) thread. The eye not fixating was occluded. The experimental arrangement was such that movements of the fixating eye did not cause any electrical artifacts which might distort the record. This is demonstrated by Figure 1, which has been taken from the previous paper.[2] In this Figure are recorded the movements of both the occluded (nonfixating) and fixating eyes, by use of the electrical recording of the ocular resting potential (ORP) from skin electrodes at the nasal and temporal canthus of each eye. The demonstration in this Figure, which was also invariably the

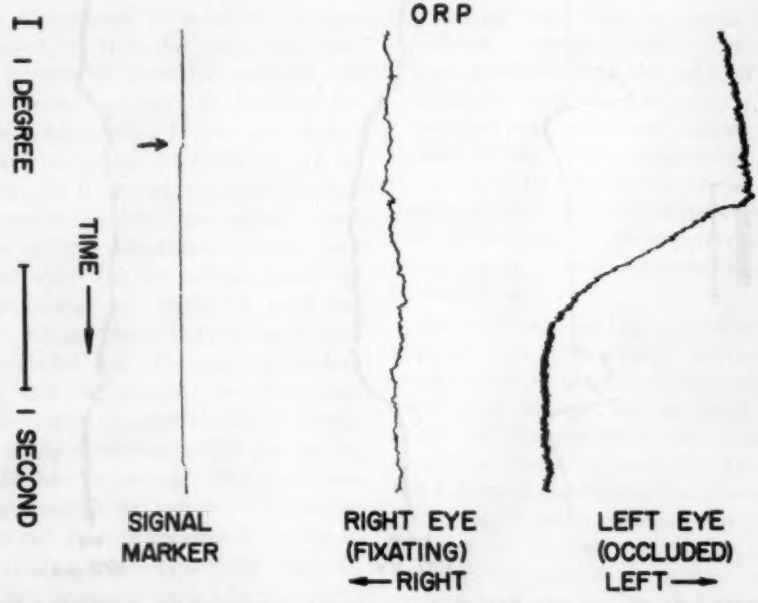

Fig. 1.—Change in the ocular resting potential (ORP) of each eye in far (340 cm.)-to-near (18.9 cm.) accommodation of the right eye. Note that no essential change in the ORP of the fixating eye occurs throughout accommodation. (Reprinted with permission from the *American Journal of Ophthalmology*.[2])

case for the observers used in the present study, is that in making the change in fixation from far-to-near the fixating eye makes *no overt movement at all.*

In the present experiments the separation of the near and far targets was such that changes in the stimulus to accommodation ranging from 1.24 to 9.5 D. were obtained. The contact lens electrode was placed onto the fixating (left) eye after the skin electrodes were located on the nasal and temporal canthi of the nonfixating (occluded) eye. The leads from the contact lens electrode and a lead from the indifferent electrode (a skin electrode placed behind the left ear) were fed into one channel of a D. C. preamplifier and recorded after amplification with pen oscillograph. The leads which recorded the ORP of the occluded eye were amplified and recorded in the second channel of the oscillograph. A third channel was used to record a voice record of the experimenter as he spoke into a microphone to give commands for change in fixation. In this way the potential recorded from the contact lens electrode during the accommodation of the fixating eye, the vergence movement of the occluded eye during accommodation of the fixating eye (such as that illustrated in Figure 1), and the stimulus artifact could all be obtained simultaneously on the record. Experiments were carried out on two young adult male observers. Although the essential details to be described have been obtained with each subject, most of the quantitative data reported will be for Subject 1, for whom more detailed data are available.

III. Results

Figure 2 illustrates the raw data obtained in a typical experiment with the contact lens electrode. In this Figure the top record is

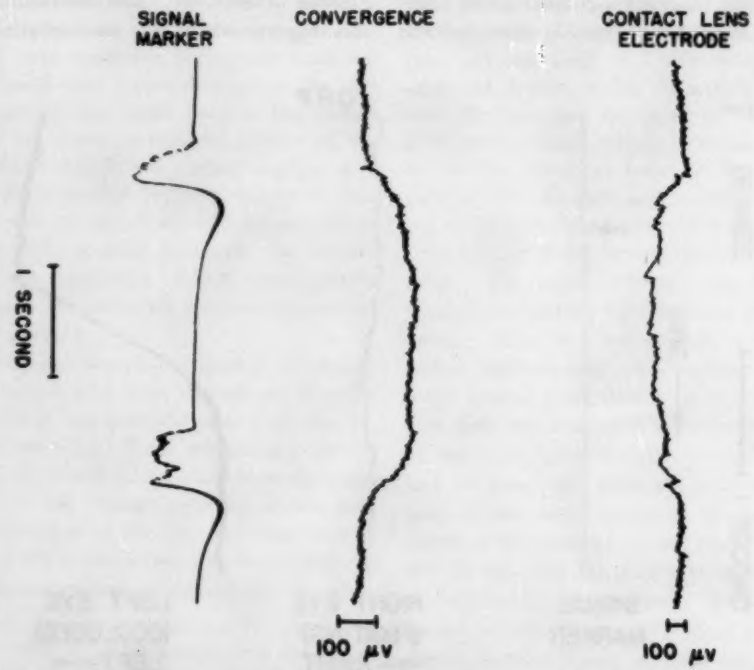

Fig. 2.—Simultaneous recording of the contact lens electrode from the fixating (left) eye and the vergence movement of the occluded eye. The left hand signal mark indicates far (192 cm.)-to-near (18 cm.); that on the right, near-to-far accommodation. Increase of positivity of the contact lens record is indicated by an upward deflection.

the potential recorded between the contact lens electrode and the indifferent electrode attached to the bone behind the left ear, the middle record represents the associated vergence movement of the occluded eye, and the bottom record represents the signal marker. The signal marker on the left indicates the response of a microphone at the instruction "near"; that on the right, the response at the instruction "far." In this experiment the near target was 18 cm. from the eye. The contact lens electrode shows an increase of negativity of about 200μv. on shift from far-to-near, which reaches a steady level in about one-third of a second. This steady level is maintained until the return to the far target, whereupon the potential returns approximately to the baseline. The corresponding vergence movement of the occluded eye (nasalward during the far-to-near shift, temporalward during the near-to-far shift) is recorded in the middle record. The relative reaction times of the responses recorded in each of the two channels are of importance for the theory of accommodation-vergence relation and will be analyzed in detail in a subsequent paper. It is to be emphasized that the ORP recorded from the occluded eye is about one-half as large as that recorded from the contact lens. If the contact lens was merely recording the ORP of the occluded eye as it moved nasalward during the change in fixation, one would expect (because of the remoteness of this lead from that eye) that the voltage picked up from the contact lens would be much less than the voltage obtained from leads next to the occluded eye. The opposite finding suggests that the potential recorded from the contact lens represents activity largely or exclusively occurring within the eye on which the lens is resting. This conclusion is strengthened by the fact that the relationship between the two voltages is not linear.

The result of the experiment just described (an invariable finding in this work) suggests that the voltage obtained from the contact lens electrode was largely generated within the fixating eye itself. One possible source of potential would be the contraction of the smooth muscle of the iris to produce the miosis of the "Naheinstellungsreaktion" (near-point reaction).

In order to investigate the role that the pupil could play in the generation of this potential, the following experiment was carried out: A strong light was presented to the contralateral eye, which permitted the pupil in the fixating eye to become so small that no observable change could be detected in the size of the pupil when the eye changed fixation from the far to the near object. The voltage measured under these conditions was then compared with that obtained when there was no light in the contralateral eye and the pupil was consequently free to move through its normal amplitude. The result of one such experiment is illustrated in Figure 3, in which the fixation distance was 20 cm. This is a histogram showing the frequency distribution of the voltages obtained in 25 separate measurements both with the light exposed to the contralateral eye and under the normal viewing conditions. It is seen that no significant difference exists between the voltage generated with the light off or on. This proved to be the case every time the experiment was performed, either in far-to-near or near-to-far accommodation, and it proved to be independent of the testing distances involved. This suggests that the role of the pupil in the generation of the voltage in far-to-near accommodation must be negligible.

The extremely unlikely possibility exists, however, that, even though no observable change could be detected in the size of the pupil when the light was on, pupil activity might still be generating the voltage obtained. In order to check this possibility, the following experiment was carried out: Photographic measurements were made of the size of the pupil when the eye was fixating the targets at various test distances, with use of normal room illumination. The contralateral eye was then exposed to a 16.4 degree adaptation field

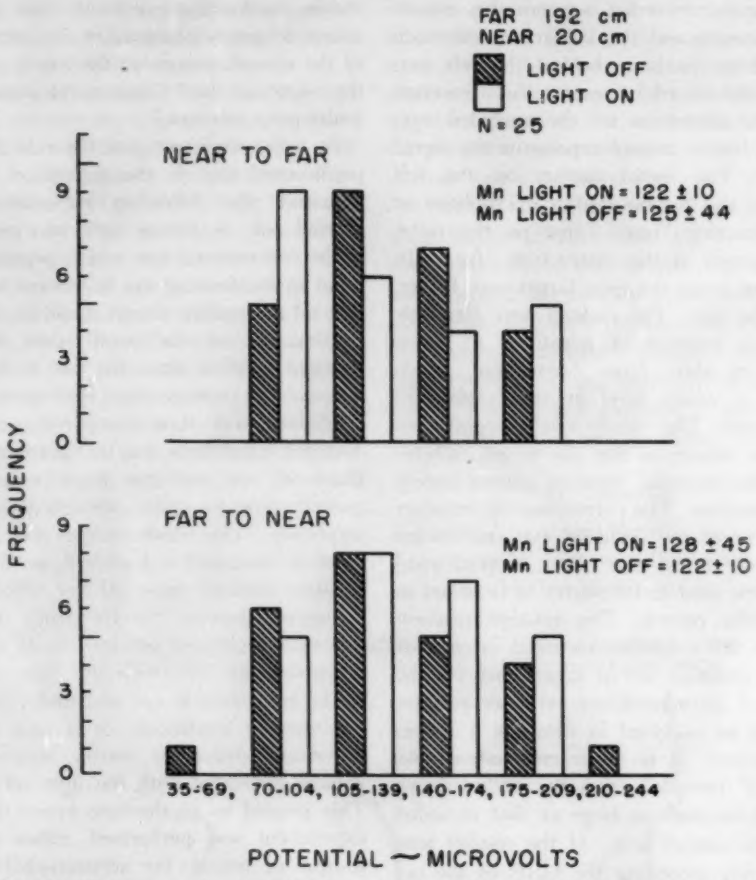

Fig. 3.—Histograms showing the frequency distribution of the voltages recorded in accommodation from far (192 cm.) to near (20 cm.) and back to far when a strong light is exposed to the contralateral eye in such a manner that no change in the size of the pupil of the fixating eye can be observed during accommodation. Control experiment for comparison is done under the usual conditions, in which the pupil size fluctuates freely.

produced by Maxwellian view. The size of the pupil was again measured in the normal room light when the contralateral eye was exposed to various field luminances. The results of both of these pupillographic experiments are illustrated in Figure 4, which depicts the pupil diameter as a function of (*a*) change in the stimulus to accommodation and (*b*) retinal illuminance of the contralateral eye. With use of this graph, it is possible to determine the retinal illuminance required in the contralateral eye to produce the magnitude of pupil constriction obtained with the maximum change in the stimulus to accommodation. The dotted lines in this Figure illustrate this determination. It is now possible to measure the magnitude of the voltage generated by the change in pupil size equal to that produced by the maximum accommodation change by exposing to the contralateral eye the relevant intensity (4×10^5 trolands) with the accommodation held fixed. The experiment proved somewhat difficult, because almost every turning on or off of the light was followed by a blink, which could be easily mistaken for a voltage generated by the pupil. If, how-

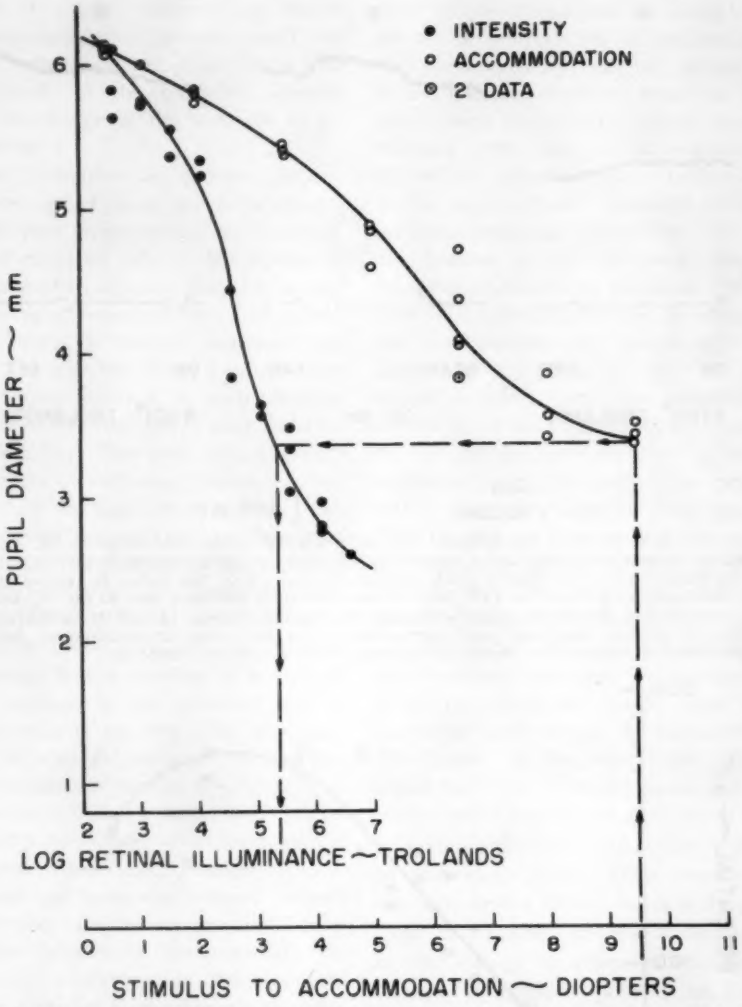

Fig. 4.—Diameter of the pupil under various stimulus intensities of light exposed to the contralateral eye and under various stimuli to accommodation. The intensity of the light required to give the same degree of pupil constriction as the maximum change in the stimulus to accommodation is recorded by the pattern of the dotted line. It is equal to 4×10^5 trolands of retinal illuminance.

ever, no blink was present, the experiment showed virtually no change in potential when the light was turned on or off. Occasionally a small change in potential (increase of negativity when the light went on and opposite change when the light went off) was just barely detectable above the noise level. Figure 5*A* shows one example of this. Figure 5*B* illustrates a control experiment showing, for comparison, the voltage generated in a shift from far-to-near (20 cm.) target. In Figure 5*C* the intensity of the light is 10 times brighter than that required for maximum pupil constriction in far-to-near accommodation, and still no obvious voltage can be detected when the light is turned off or on.

The experiments described above seemed to indicate that the potentials recorded from the contact lens electrode in changing fixa-

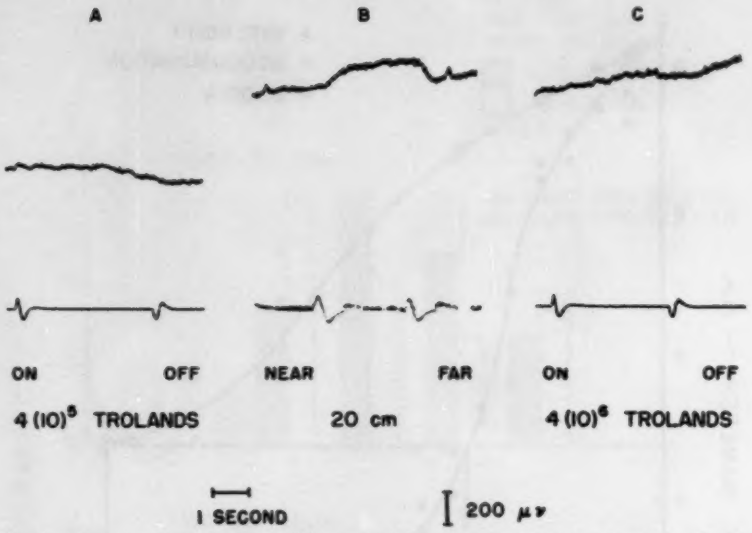

Fig. 5.—Raw record of the potential recorded from the contact lens electrode. *A*, stimulus to accommodation held fixed and a bright (4×10^6 trolands) light exposed to the contralateral eye by Maxwellian view. Signal mark indicates on and off of this light. *B*, control experiment accommodation from far (192 cm.) to near (20 cm.) and then back to far. *C*, same as in *A* except that the photic stimulus is now 10 times as intense (4×10^6 trolands) as that required to produce maximum pupil constriction in the far-to-near accommodation. Increase of positivity of contact lens record is indicated by a downward deflection.

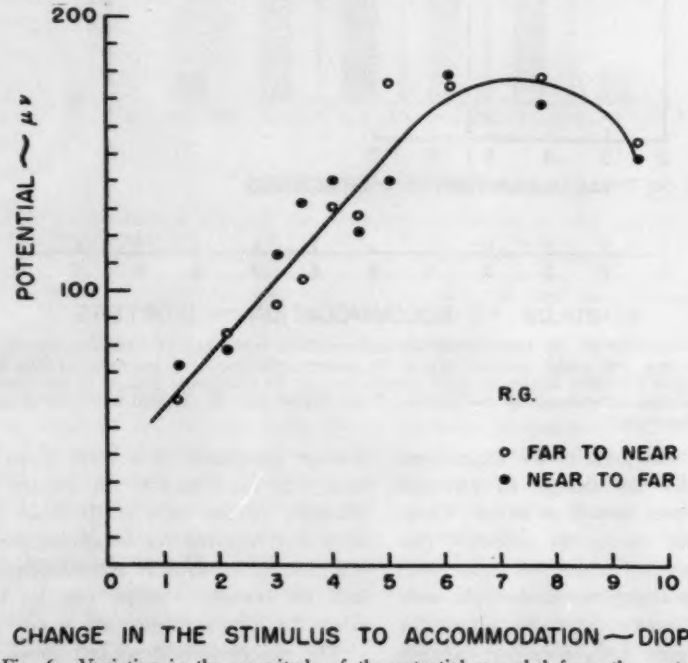

Fig. 6.—Variation in the magnitude of the potential recorded from the contact lens electrode as a function of the change in the stimulus to accommodation. The results are the means of two repetitions of the experiment.

tion from far to near were closely related to the accommodation process itself. It therefore seemed important to know how the magnitude of the recorded potential varied with change in the stimulus to accommodation.

In order to answer this question, experiments were carried out in which the separation of the near target was varied randomly through a range of values. Measurements were made on two separate days, the second session being a repeat of the first except that the order of testing distances was changed. On each day from 5 to 30 measurements were obtained at each distance both in the shift from far to near and that from near to far. The same general trends were apparent on each day; therefore, only the means of the data are presented. The results of this experiment are illustrated in Figure 6, which shows the variation of the recorded potential as a function of the change in the stimulus to accommodation. It is seen in the Figure that the increase in the change in the stimulus is associated with an increase in the potential until a maximum value is reached. It is important to point out that the value of the abscissa at the maximum ordinate in this figure is in good agreement with the value (6.57 D.) of the subject's amplitude of accommodation as measured in the clinic. Further increase in the stimulus to accommodation beyond this maximum value is not associated with any further increase in the potential. On the contrary, a decrease in the measured voltage is obtained at the highest stimulus levels. These data have important theoretical implications for the operation of accommodation and the theory of presbyopia, which are the subject of further studies to be published soon. For the present purpose it will suffice to point out the general relation between change in the stimulus to accommodation and the magnitude of the potential which has been found.

How does the magnitude of this potential vary with changes in the actual amount that the eye accommodates? Technically, this question is difficult to answer because some suitable means must be found to measure the accommodation of the eye, preferably simultaneously with the measurements of the potential recorded from the contact lens. Some preliminary results have been obtained with use of a stigmatoscope, viewed by reflection from a half-silvered mirror, and a Badal optometer to measure the accommodation subjectively. The essential features of the apparatus have been described elsewhere by one of us (M. A.).[3] The only additional feature here employed was a cam-operated microswitch which flashed the stigmatoscope light on for one second in every two, thus preventing the subject from accommodating on the stigma. All of the electrical features of the stigmatoscope were shielded. The procedure was to have the observer look through a 3.00 D. concave lens at the fine scale of a white meter stick, mounted at 116 cm. distance from his eyes. He was able to clear the target or to blur it on command, and the voltage from the contact lens was simultaneously recorded. A setting on the Badal optometer was made prior to and following each change in accommodation. The results of the experiment are illustrated in Figure 7, which shows the potential recorded for various amounts of change in accommodation. Each point represents an individual datum. The smooth curve has been drawn merely to show the general trend of the data. There is a clear increase in the voltage as the accommodation increases. It should be pointed out, however, that this experiment permitted only a small range of accommodation values to appear; consequently, no theoretical interpretation of such data will be attempted at this time, beyond the obvious verification of the fact that the potentials are related to actual changes in focus of the eye. In a subsequent paper the relation between these two variables will be subject to further analysis, over a wider range of values, and the theoretical implications of these data and those in Figure 6 will be discussed in some detail. For the present purpose, it will suffice to

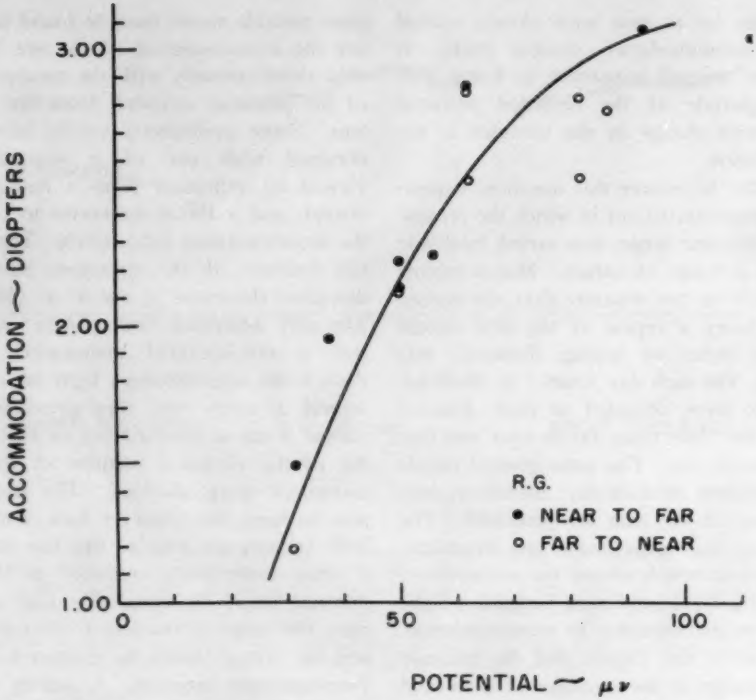

Fig. 7.—Variation in the magnitude of the potential as a function of the variation in the amount of change in the accommodation of the eye. The latter data were measured by viewing a Badal optometer and a stigmatoscope through a half-silvered mirror while the eye accommodated or relaxed through a —3D. lens. Each point is an individual datum.

point out that there is good agreement between these two studies.

Comment

The experiments demonstrate that there is a close quantitative relationship between the magnitude of the potential recorded from an electrode over the sclera in the region of the ciliary muscle during the process of accommodation and the magnitude of the accommodation response. It seems reasonably certain that the potential is not generated by the movements either of the fixating eye or of its occluded fellow or by contraction and relaxation of the smooth muscle of the iris during accommodation. Is it possible to say more as to the source of this potential?

It has been suggested by Noell [4] that the process of accommodation, the contraction of the ciliary muscle with its mechanical traction on the choroid, and the abrupt change in form of the lens surfaces and of the zonule are capable of producing marked D. C. changes in the resting potential existing between the front and the back of the eye. This change of potential would be similar to what can be recorded between a needle electrode at the limbus and an indifferent electrode at the back of the eye as the needle is suddenly thrust into the globe. In order to test this proposal, we have placed a EEG skin electrode on the external canthus as close to the eye as we could get it. Records were made of the potential picked up between this electrode and an indifferent electrode on the skin behind the ear. At no time was it possible to pick up any change in potential between these two electrodes when the eye changed focus from a far to a near target. This was

true even though the stimulus was such that the contact lens electrode would have picked up a near maximum voltage and even though the apparatus was capable of easily detecting 5μv. signals from the noise. Bornschein and Schubert [5] have recently measured the magnitude of the resting potential recorded for a given size eye movement (*a*) when the eye is fixating at far and (*b*) when it is accommodating for near. They were unable to find any difference in the magnitude of the voltages recorded in these two different conditions. These two experiments are contrary to what might have been expected if Noell's theory were correct, although neither of them can be considered crucial.

Schubert [1] and Bornschein and Schubert [5] believe that the contact lens is recording an "integrated" action potential generated by the ciliary muscle whenever the eye accommodates or relaxes its accommodation. The polarity of the potential supports this notion. With the electrode described in this paper the far-to-near accommodation is always accompanied by an increase of negativity of the electrode, while looking from near to far is always accompanied by an increase of positivity of the electrode. Schubert described the opposite polarity changes. This discrepancy can be attributed to difference in the geometry of the electrode and their respective localizations with respect to the eye. For example, if a standard ERG electrode is used, in which contact is made with the cornea, then it can be shown (under conditions in which no change in pupil diameter occurs) that the accommodation is associated with an increase in *positivity* of the contact lens electrode. The variation in polarity as the position of the electrode is changed with respect to the eye is exactly what would be anticipated if the lens electrode were picking up a highly localized action potential generated by the muscle itself.

Nevertheless, many more data will be required before a definite decision can be made about these matters. It is true [6-10] that the single smooth muscle cells have recently been shown to have a membrane potential and that the contraction of such a muscle cell in physiological preparation can be associated with high frequency "spike" action potentials. It remains to be shown, however, first, that such electrical phenomena occur in the mammalian ciliary muscle cell and, second, the way these electrical changes from single cells integrate to produce the slow D. C. waves recorded from the contact lens electrode. Clearly, a great deal of precise physiological experiments on whole ciliary muscle and single cells need yet to be done before one can unequivocally adopt Schubert's hypothesis.

It is also possible that the potential may be produced by some other process which changes during accommodation that has not yet been given detailed consideration. Two such processes readily come to mind. One of these is the obvious changes in the form of the lens which are an essential feature of accommodation Brindley [11] has shown that the inside of the lens is 70 mv. more negative than its surroundings. It seems possible that changes in this voltage would occur during accommodation. It is also possible that changes in blood volume [12] of the ciliary body might occur during accommodation and that this could be responsible for the effect. Clearly, a considerable number of purely physiological experiments will be required before the source of the D. C. change in potential which occurs during accommodation can be established with certainty.

Summary

A relatively safe and simple technique is presented which permits measurement of the D. C. shift in potential in the human eye that is generated in accommodation of the eye from far to near or from near to far. Experiments demonstrate that movements of either the fixating or occluded eye or contraction of the smooth muscle of the iris is not responsible for the effect. The way in which this potential varies over a range of changes in the stimulus to accommodation and the actual changes in accom-

modation of the eye are described. A considerable number of further experiments will be required to establish with certainty the source of this potential.

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Penetration of Pyrimethamine (Daraprim) into Ocular Tissues of Rabbits

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Chemically, pyrimethamine (Daraprim) is 2,4-diamino-5-*p*-chlorophenyl-6-ethylpyrimidine, with the empirical formula $C_{12}H_{13}N_4Cl$. This chemical was synthesized by the Wellcome Research Laboratories, Tuckahoe, N. Y., as an antimalarial drug which arrests plasmodial development at those stages involving nuclear division. Besides this antimalarial activity, pyrimethamine has been shown to have an extraordinarily potent antimetabolite activity.

To my limited knowledge, the mode of action of the preparation against toxoplasmosis has not been entirely elucidated. However, Eyles and his associate,[1] in 1952, reported antitoxoplasminic action of the drug in mice and, in 1953, showed that pyrimethamine and sulfadiazine were synergistic to each other against experimental toxoplasmosis in the mouse.[2] Since these reports, the drug has been widely used in the treatment of presumptive toxoplasmic infections of man as well as of experimental toxoplasmosis of animals.

In the past years, numerous papers on the subject have been published, creating two major conflicting currents of opinions as to the effectiveness of the drug against ocular toxoplasmosis. The conflict may be attributed to these several components: 1. There is difficulty in establishing concrete diagnosis of toxoplasmic lesions in human eyes. Even when there is an established diagnosis of ocular toxoplasmosis, the dual pathogenesis of lesions, some being due to cyst rupture and some to proliferative forms of Toxoplasma, must be clarified as far as the therapeutic potentiality of pyrimethlamine is concerned. 2. The nature and dose of other drugs, such as steroids and sulfonamides, when administered along with pyrimethamine are another source of confusion in evaluating the effectiveness of pyrimethlamine against human ocular toxoplasmosis. 3. Finally, the dose of pyrimethamine used in the treatment of ocular toxoplasmosis varies from one author to another, ranging from 25 to 75 mg. a day, and the duration and interval of medication also vary, in addition to the variation in dosages of subsidiary or synergistic drugs mentioned above. Considering these three components, it is highly advisable to standardize the therapeutic dose of pyrimethamine in order to evaluate its effectiveness against ocular toxoplasmosis.

Cook and his associate,[3] using eight-day-old roller-tube cultures of minced mouse embryonic tissues inoculated with Toxoplasma gondii of the R. H. strain, have demonstrated that 1μg. of pyrimethamine per cubic centimeter prevented the multiplication of T. gondii organisms but did not destroy them and that 10μg. of pyrimethamine per cubic centimeter completely destroyed T. gondii. On the basis of their experimental work they concluded that "the lowest effective concentration of pyrimethamine (Daraprim) in these tissue culture tests indicates that this lies in the range of three or four micrograms per cubic centimeters."

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Yonsei University, School of Medicine.

Dr. E. N. Whitman, Burroughs Wellcome & Company, Inc., provided the pyrimethamine powder.

This work was done at the Wills Eye Hospital, Research Department, at the suggestion and under the guidance of Dr. Irving H. Leopold, the Director.

In view of these facts, studies of the penetration of the drug into ocular tissues have been carried out in order to estimate the dose of pyrimethamine sufficient to meet this criterion in rabbit eyes.

Experimental Method

The paper-disc assay method was used, with use of Streptococcus faecalis, A. T. C. C. 8043, as an indicator.[4] Chinchilla rabbits were used, equal numbers of males and females, weighing 2.5 to 4.0 kg.

Preparation of Inoculum.—S. faecalis, cultured overnight in broth (yeast extract 1%, glucose 1%), was centrifuged and washed four times with saline in order to remove traces of folic acid and was then suspended in saline until the mixture was faintly cloudy.

Preparation of Seed Plates.—Bacto-Folic Acid Assay medium 7.5 gm., of folic acid, 0.1μg., and agar, 2 gm., were dissolved in 100 cc. of distilled water, and the preparation was autoclaved. When the temperature fell to about 45 C, 50 cc. of inoculum prepared previously was poured into the medium. The mixture was shaken in such a way that the cells could be evenly distributed throughout the container and then was poured into Petri plates, about 15 cc. in each plate.

The seed plates so prepared were kept in a refrigerator. They were prepared once a week to avoid the error which might have happened by the growth of S. faecalis in the seed plate during storage.

Method of Assay.—Filter-paper discs, 10 mm. in diameter, were placed upon the surface of the seed plate; 0.03 cc. of specimens, aqueous humor, vitreous humor, tissue extracts, and standard pyrimethamine solutions were added to separate discs. The plate was incubated overnight, and the zones of inhibition were measured with a millimeter ruler.

Pyrimethamine solutions with known concentrations were tested simultaneously each time an assay was run, since conditions of autoclaving and temperature of incubation cannot be duplicated exactly from time to time.

Intravenous Injections

1. *Pyrimethamine Concentration in Serum.*—It was believed that the concentration of pyrimethamine in serum had to be estimated before the concentration in the ocular tissues was checked in order to correlate the presence of the drug in the blood stream and in the ocular tissues.

Ten rabbits were used in this study and were divided into five groups of two rabbits each. Rabbits in each group received an intravenous pyrimethamine injection of 8, 6, 4, 2, and 1 mg. per kilogram of body weight, respectively. Then blood was drawn at 15, 30, 60, 120, and 240 minutes, respectively, and sera were obtained. Figure 1 shows the average values of two readings in each group.

One of the rabbits receiving 8 mg. per kilogram died in convulsions within 15 minutes after the injection, and the sera

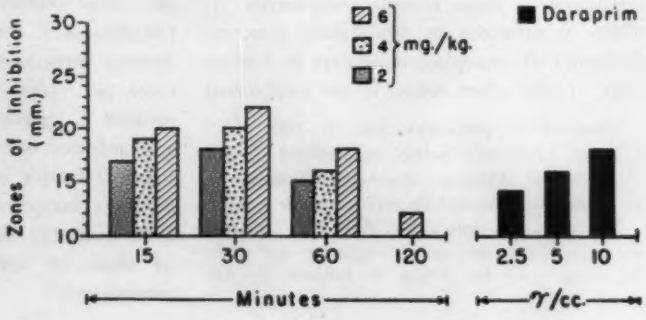

Fig. 1.—Concentration of pyrimethamine in the blood stream of normal rabbits after intravenous injection. The concentration is represented by the zones of inhibition of S. faecalis growth and compared with the zones of inhibition of known concentrations of pyrimethamine.

from rabbits receiving 1 mg. per kilogram showed no inhibitory action at all. These two groups are not shown in the Figure.

The blood level rose rather abruptly within 15 minutes after the injection and fell sharply within 2 hours.

2. *Pyrimethamine Concentration in the Aqueous and Vitreous.*—In normal undisturbed eyes: Twelve rabbits received an intravenous injection of pyrimethamine, 2 mg. per kilogram of body weight. The rabbits were divided into four smaller groups of three each. Prior to the sample-taking, eyes were anesthetized with 2 drops of 0.5% tetracaine hydrochloride solution, were flushed with sterile isotonic saline, and were proptosed with the aid of a muscle hook by an assistant. For the tapping of the aqueous, a sterile 27-gauge needle and a sterile tuberculin syringe were used; for the tapping of the vitreous through the sclera behind the lens a 20-gauge needle was used. The aqueous and vitreous of the rabbits in the different groups were tapped at 30, 60, 120, and 240 minutes.

The concentration of pyrimethamine in micrograms per cubic centimeter in the aqueous and vitreous was determined by the paper-disc method previously described.

The same procedure was repeated on another group of rabbits receiving 4 and 6 mg. per kilogram, respectively.

Pyrimethamine was not detectable in the aqueous and vitreous in any of those groups tested.

In Disturbed Eyes: The penetration of a systemically administered drug into the eye depends primarily upon the permeability of the blood-ocular barriers. Studies were therefore conducted to determine the effect of inflammation and double tap upon the penetration of pyrimethamine into the eye.

Inflamed eye studies. Approximately one-half of the right cornea of the six rabbits was burned with hydrochloric acid (2 N). The rabbits were divided into three groups of two each. One hour after the burning, intravenous injections of pyrimethamine were made: The first group received 6 mg. per kilogram; the second group, 4 mg. per kilogram; the third group, 2 mg. per kilogram of body weight, respectively. One hour after the injections, two hours after the burnings, the aqueous and vitreous of both eyes were tapped and subjected to the filter-paper-disc assay. The results are presented in Table 1. Only the 6 mg. group showed slight evidence of penetration of the drug into the aqueous but not into the vitreous.

TABLE 1.—*Concentration of Pyrimethamine in the Aqueous Humor of Chemically Inflamed Rabbit Eyes After Intravenous Injection* *

Pyrimethamine dose, mg/kg.	Zone of Inhibition, Mm.	
	Inflamed Eye	Intact Eye
6	12	0
4	0	0
2	0	0

Known concentration of pyrimethamine, γ/cc.	
10	17.7
5	16.5
2.5	13.5

* The concentration is represented by the zones of inhibition of S. faecalis growth and is compared with the zones of inhibition of known concentrations of pyrimethamine.

Double-tap studies. Three rabbits each received an intravenous injection of pyrimethamine, 6 mg. per kilogram of body weight. The aqueous and vitreous of both eyes were tapped twice, once at the end of 30 minutes and once at the end of 60 minutes. The results of the filter-paper-disc assay are shown in Table 2. It can be seen that a previous paracentesis slightly accelerated the penetration of the drugs into the anterior chamber but not into the vitreous cavity, even when a massive dose of drug, close to the margin of safety in rabbits, was administered.

3. *Uveal Tissue Studies.*—Despite the rapid rise of concentration in the blood stream following intravenous injection, no appreciable amount of pyrimethamine was demonstrated in the ocular fluids. Assuming that the blood-aqueous and blood-vitre-

TABLE 2.—*Concentration of Pyrimethamine in the Secondary Aqueous Humor of Normal Rabbit Eyes After Intravenous Injection* *

Animal No.	Zone of Inhibition, Mm.							
	Aqueous				Vitreous			
	30 Min.		60 Min.		30 Min.		60 Min.	
	O. D.	O. S.	O. D.	O. S.	O. D.	O. S.	O. D.	O. S.
70	0	0	12	12	0	0	0	0
71	0	0	14	Trace	0	0	0	0
72	0	0	14	14	0	0	0	0

Known concentration of pyrimethamine, μg/cc.	
10	17.7
5	16.5
2.5	13.5

* The concentration is represented by the zones of inhibition of S. faecalis growth and is compared with the zones of inhibition of known concentrations of pyrimethamine.

ous barriers have selective activity in permeability, one can easily presume that the drug in question may be held in the uveal tissue but may not be allowed to penetrate into ocular chambers. To make it clear whether or not this presumption is true, uveal tissues were extracted with acetic acid and the extracts were subjected to the filter-paper-disc assay. The procedures were as follows: All manipulations were carried out under sterile conditions. Eyes were enucleated from the pyrimethamine-treated rabbits and put into a sterile glass bottle and kept in a refrigerator until they were subjected to the dissection. In dissection, a crucial section of the cornea was made; iris and ciliary body were easily detached from the choroid at the pars plana by pulling the iris with a pair of toothed iris forceps. The crucial sections of the cornea were extended backward beyond the equator in four directions, and lens, vitreous, and retina were removed with the aid of cotton applicators and a pair of iris scissors. The choroid was stripped off of the sclera by blunt dissection, with use of Daviel's lens spoon. The removed irises and ciliary bodies of two eyes were mounted on a cover glass; the choroids of the same eyes were mounted on another cover glass and were weighed separately. In extraction, 3 cc. of acetic acid (0.1 N) was added to every 1 gm. of wet uveal tissues. These preparations were ground in a mortar with Alundum and were centrifuged. The extracts so obtained were made available for the filter-paper-disc assay.

A single dose of intravenous injection of pyrimethamine, 4 mg. per kilogram of body weight, was given to eight animals, which were divided into four groups of two each. The rabbits were killed by air injection. The time intervals at which the rabbits were killed were 30, 60, 120, and 240 minutes, respectively.

The same procedure was repeated on another group of rabbits, which received 2 mg. per kilogram of body weight.

The average values of two readings of the zones of inhibition are shown in Figure 2. The intrauveal level of the drug following a single intravenous injection rose rapidly within 30 minutes to more than 5μg. per cubic centimeter and was maintained at this level about 2 hours. The concentration of the drug in the anterior

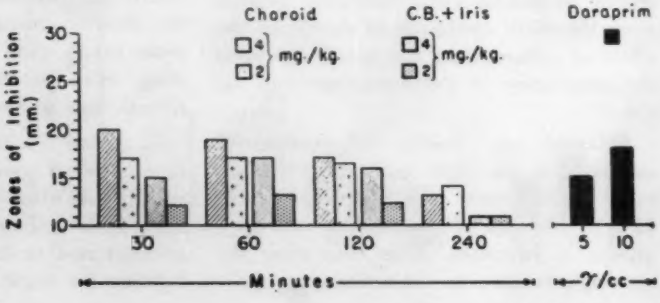

Fig. 2.—Concentration of pyrimethamine in the uveal tissue of normal rabbit eyes after intravenous injection. The concentration is represented by the zones of inhibition of S. faecalis growth and compared with the zones of inhibition of known concentrations of pyrimethamine.

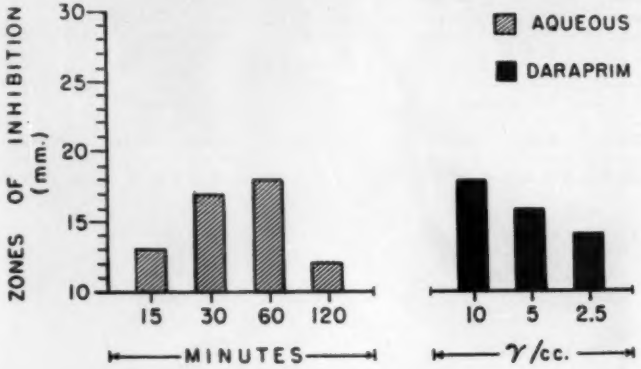

Fig. 3.—Concentration of pyrimethamine in the aqueous humor of normal rabbit eyes after subconjunctival injection. The concentration is represented by the zones of inhibition of S. faecalis growth and compared with the zones of inhibition of known concentrations of pyrimethamine.

uvea (the iris and ciliary body) was higher than in the posterior uvea (the choroid).

Subconjunctival Injections

After the instillation of 0.5% tetracaine hydrochloride, 15 rabbits received a single dose of bilateral subconjunctival injection of pyrimethamine, 0.05 mg. in a 0.5 cc. volume. The rabbits were divided into five groups of three each. The aqueous and vitreous of both eyes were tapped with the same technique as described before. The time intervals at which the specimens were obtained were 15, 30, 60, 120, and 240 minutes after the subconjunctival injections of pyrimethamine. The concentrations of pyrimethamine in the specimens are shown in Figure 3. Each figure represents the average value of six eyes of three rabbits.

Pyrimethamine level in the aqueous humor after a single subconjunctival injection was satisfactory even with a small dose of pyrimethamine and fell rather rapidly in less than two hours. However, the concentration of pyrimethamine in the vitreous in the same period of time was nil, which is not presented in the Figure.

Retrobulbar Injections

Twelve rabbits were chosen for this study. Eyes were anesthetized with 2 drops of 0.5 tetracaine hydrochloride solution. Bilateral retrobulbar injections of pyrimethamine, 0.1 mg. in 1 cc., were given. Rabbits were divided into four groups. The technique of sampling and assay was the same as previously described. The time intervals at which the samples were taken were 15, 30, 60, and 120 minutes after the injection.

The concentrations of pyrimethamine in ocular fluids after retrobulbar injections are charted in Table 3. As one may see in the Table, pyrimethamine level in the aqueous is not consistent but, rather, has a sporadic pattern. On reviewing the notes made during experiments, it was found that the eyes showing a satisfactory concentration of the drug in the aqueous had a good bleb after the retrobulbar injections simulating subconjunctival injections. Pyrimethamine level in the vitreous is practically nil. Therefore, one may conclude that pyrimethamine injected into retrobulbar spaces may not penetrate into ocular chambers at all, or very little, unless it floods into subconjunctival spaces. The over-all picture of this study indicated that pyrimethamine injected into the retrobulbar space did not penetrate into the ocular chambers but did confirm that the subconjunctival injection was an adequate route to permit entry of pyrimethamine into the aqueous.

Tolerance Studies

1. *Intravenous Injections.*—During the series of experiments some animals experienced a transient convulsion even with

TABLE 3.—*Inconsistent Appearance of Pyrimethamine in the Aqueous Humor of Normal Rabbit Eyes After Retrobulbar Injection* *

	Zone of Inhibition, Mm.															
	Aqueous								Vitreous							
	15 Min.		30 Min.		60 Min.		120 Min.		15 Min.		30 Min.		60 Min.		120 Min.	
Animal No.	O. D.	O. S.	O. D.	O. S.	O. D.	O. S.	O. D.	O. S.	O. D.	O. S.	O. D.	O. S.	O. D.	O. S.	O. D.	O. S.
61	0	0							0	0						
62	0	14							0	0						
63			13	0							0	0				
64			13.5	14							0	0				
65					0	0							0	0		
66					16	0							0	0		
67							14	14							0	0
68							0	0							0	0
Known concentration of pyrimethamine, μg/cc.																
10								17.7								
5								16.5								
2.5								13.5								

* No pyrimethamine was detectable in the vitreous humor of the same eyes.

an intravenous dose of 4 mg. per kilogram of body weight. One of two rabbits died immediately after having an intravenous injection of pyrimethamine, 8 mg. per kilogram of body weight, in the study of blood concentration of pyrimethamine. To make sure that this death was due to overdose of pyrimethamine, another five rabbits were chosen to receive an intravenous injection of pyrimethamine, 8 mg. per kilogram of body weight. Of those five rabbits, three died within 15 minutes in convulsions; two of them experienced a transient convulsion. The rabbits that survived received an additional 8 mg. of pyrimethamine per kilogram and died in convulsions. It is not the purpose of this paper to determine the lethal dose of the drug. However, the data strongly indicate that a single intravenous injection of 8 mg. per kilogram of body weight is well over the margin of safety in rabbits.

2. *Subconjunctival Injections.*—Three normal adult rabbits were chosen for this study. Each eye of the rabbits received a daily subconjunctival injection of pyrimethamine, 0.05 mg. in a 0.5 cc. volume, with use of 0.5% tetracaine anesthesia. External examinations were made daily during the first week, and pupils were dilated for detailed observation at the end of each week. No significant local reactions were noticed except an occasional mild congestion around the site of injection.

One of the rabbits was killed by air embolism at the end of the 4th week; the second, at the end of the 6th week, and the last, at the end of the 10th week. Eyes were enucleated and prepared for histopathological study. Microscopic examination revealed that negligible to mild degree of cyclitic membrane was present in all of the eyes examined, and the thickness of the cyclitic membrane paralleled the duration of the treatment. No evidence of cataract was found. The findings indicate that all of the animals so treated had, more or less, a subclinical inflammation in and around the ciliary body during the treatment (Fig. 4).

3. *Intracameral Injections.*—Right eyes of three normal adult rabbits were subjected to these studies. After instillation of 0.5% tetracaine into the eyes, the aqueous humor was withdrawn and 0.2 cc. of pyrimethamine solution containing 10 mg. per cubic centimeter was injected into the anterior chamber with sterile technique. The eyes were examined every day for a week. Twenty-four hours later the lids were swollen and reddened, the conjunctiva was chemotic and congested, and the cornea was

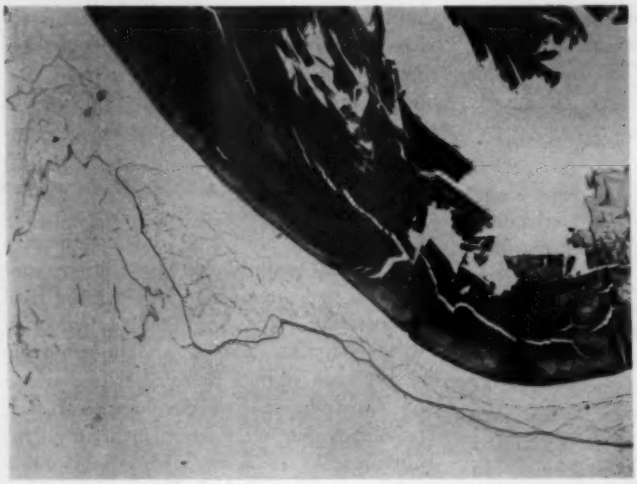

Fig. 4.—A mild cyclitic membrane after subconjunctival injection of pyrimethamine for two months.

edematous; the thickness of the cornea was almost three times that of normal cornea; the endothelial layer was hardly visible. A strong reaction was noticed in the anterior chamber, characterized by numerous cells, mostly pigment cells; fibrinous exudates, and a strong beam. The anterior surface of the lens was almost completely covered with a pigment sheet which did not permit the further examination of the posterior segment of the eye.

Eyes were enucleated at the end of the eighth week for histopathological examination. Microscopic examination revealed that there were moderate to marked sequelae of keratitis and anterior uveitis and mild to moderate sequelae of posterior uveitis but no evidences of cataractous changes.

4. *Intravitreal Injections.*—Six normal adult rabbits were chosen for these studies, and the right eyes received an intravitreal injection of pyrimethamine with sterile technique. The first three received 5.0 mg. in 0.5 cc. of solution; the second three received 0.5 mg. in 0.5 cc. At the end of 24 hours after the injections, the eyes given injections of 5 mg. of the drug showed marked opacity of the vitreous which was so dense that the fundus was not visible. The injection of 0.5 mg. produced a moderate number of floaters in the vitreous, and the retina seemed to be edematous and structureless. By the end of the first week the eyes given injections of 5 mg. of the drug showed dense opacity of the vitreous which still did not allow the clear view of the fundus. The eyes given 0.5 mg. injections showed a fairly clear vitreous which permitted one to see the detached and fragmented retina in the fundus. The first group, 5 mg. group, was killed on the ninth day. Eyes were prepared for histologic examination.

Microscopic examination revealed that all of the three eyes developed retinal detachment, and the detached retina was so disintegrated that the normal structures of the retina were not recognizable. In addition to these findings, there were signs of acute uveitis. One of three eyes in the group developed posterior subcapsular cataract. The 0.5 mg. group was kept for five weeks and examined weekly. All three eyes showed white bands in the vitreous running in and anteroposterior direction with whitish appearance of the retina. Two of the three eyes developed a mild posterior synechia. One of them developed a very mild posterior subcapsular opacity. At the end of the fifth week, all three rabbits were killed. The eyes were prepared for histologic examination. Microscopic studies conformed the clinical findings—mild to moderate cyclitic

membrane and mild disintegration of the retina in three, posterior synechia in two, and posterior subcapsular cataract in one eye.

Comment

It is evident from the data that single intravenous injections of pyrimethamine, regardless of dose, did not penetrate either into the aqueous humor or into the vitreous humor of normal rabbit eyes, despite the acute rise in blood level following the injection. However, a small amount of pyrimethamine was detected in the aqueous of the eyes which were previously burned with hydrochloric acid and also in the secondary aqueous humor in double-tap studies. In any case, there was no evidence of penetration of the drug into the vitreous humor.

Pyrimethamine appeared to be present in appreciable concentration in the uveal tissues of normal rabbits after the injection of 2 mg. per kilogram of body weight. In the studies of the uveal concentration of the drug it is fair to assume that the uveal contents were somewhat diluted while the assay was proceeding, since 3 cc. of 0.1 N acetic acid was added to every 1 gm. of wet uveal tissues in the extraction. Therefore, it would appear that a single intravenous injection of 2 mg. per kilogram of body weight might produce adequate therapeutic concentration of pyrimethamine in the uveal tissues for two hours, and more likely so in uveitis. However, a satisfactory therapeutic level against ocular toxoplasmosis has not been established as yet. Such a level will depend largely on the sensitivity to pyrimethamine in vivo of the strain of the Toxoplasma and on the pathogenicity of the lesion in the eye. The present study simply indicates the concentrations that can be obtained in the uveal tissues and aqueous humor of normal rabbit eyes.

The data given here have shown that a single subconjunctival injection of 0.05 mg. in 0.5 cc. penetrated into the aqueous humor easily, raising the level up to more than 5μg. per cubic centimeter for an hour. At the same time, the drug did not enter the vitreous humor. Although prolonged continuation of subconjunctival injection produced no apparent clinical side-effects, histologic examination revealed the presence of subclinical uveitis.

Smith [5] reported that, although the strains (T. gondii) used were highly sensitive to pyrimethamine, the drug did not modify experimental toxoplasmic choroiditis in animals and that attempts to increase the local concentration by subconjunctival injection led to cataracts. The data obtained from the tolerance studies revealed that 2 of 13 eyes carefully studied developed cataract. Those eyes that received an intravitreal injection of 0.5 to 5 mg. of pyrimethamine in 0.5 cc. showed clinically a marked irritative reaction and histologically a heavy cyclitic membrane and a detached disintegrated retina. It is reasonable to assume, therefore, that the cataract formation is part of the total destructive effects of massive doses of topically applied pyrimethamine and does not reflect the specific action of the drug. Indeed, it is conceivable that the cataract may be secondary to the uveitis, which may be induced either by the chemical irritation of pyrimethamine or by the toxic effect of the products of the offending organism.

In any case, the subconjunctival injection of 0.05 mg. of pyrimethamine in 0.5 cc. did not produce a cataract.

Summary

Intraocular penetration of pyrimethamine (Daraprim) in the rabbit eye was studied by paper-disc-assay method, with use of Streptococcus faecalis as an indicator.

With a single intravenous injection of pyrimethamine a prompt satisfactory blood level was obtained within 15 minutes and this level acutely fell off in less than 2 hours.

Regardless of the single dose given, no pyrimethamine was detected in the aqueous humor and vitreous humor of normal rabbit eyes, but a trace of pyrimethamine was detected in the secondary aqueous humor and

in the aqueous humor of chemically inflamed rabbit eyes.

A dose of 2 mg. per kilogram of body weight gave an appreciable level of pyrimethamine in the uveal tract within 30 minutes, and this level was maintained more than 120 minutes after the injection.

A single subconjunctival injection of pyrimethamine solution, containing 0.05 mg. in 0.5 cc., readily penetrated into the aqueous humor but not into the vitreous humor of normal rabbit eyes.

A single retrobulbar injection of pyrimethamine solution, containing 0.1 mg. in 1 cc., did not penetrate into the aqueous humor and vitreous humor of normal rabbit eyes.

Six milligrams of pyrimethamine per kilogram of body weight was the maximum safety dose in the rabbits when it was given intravenously as a single dose.

Prolonged subconjunctival injection therapy with pyrimethamine solution containing 0.05 mg. in 0.5 cc. produced subclinical uveitis but did not cause cataract.

A single intravitreal injection of pyrimethamine solution, 0.5 to 5 mg. in 0.5 cc., caused posterior subcapsular cataract in two of six eyes treated. All six eyes so treated suffered severe endophthalmitis.

Dr. Theodore W. Sery gave advice in the bioassay method, and Mrs. Maria Patti Notaro, H. T., prepared the pathologic slides.

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Visual Functions in Patients with Retinal Pigmentary Degeneration Following the Use of NP 207

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Introduction

During the year 1956 there appeared in the ophthalmic literature two reports concerned with toxic effects produced upon the retina by a new drug belonging to the group of so-called tranquilizers (Verrey,[1] Goar and Fletcher[2]). The new drug, 3-chloro-10-[2-N-(methylpiperidyl)ethyl] phenothiazine, designated by its producer, Sandoz Pharmaceutical, as NP 207, was developed in an effort to reduce the systemic effects of chlorpromazine hydrochloride. This goal was, indeed, achieved, and the tranquilizing effect of NP 207 proved to be as good as, or better than, that of chlorpromazine, from which it was derived. However, it soon appeared that NP 207 caused very undesirable visual disturbances. Subjectively they expressed themselves in a more or less severe impairment of dark adaptation and in a loss of visual acuity. Objectively one found in the early stages a hyperemia of the disc and some edema of the retina and in the later stages more or less severe pigment disturbances of the retina. The appearance of the pigment varied; one rather characteristic and unusual form was the distribution of the pigment in large homogeneous sheets with scalloped edges toward the periphery.

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Department of Ophthalmology, College of Medicine, State University of Iowa (Dr. Burian); Department of Ophthalmology, Baylor University College of Medicine (Dr. Fletcher).

It must be assumed that the dosage of the drug played a considerable role in the production of the retinal disturbances, since some observers reported these disturbances in a rather high percentage of the cases, whereas others did not observe them at all (Rintelen,[3] Heim,[4] Rucker[5]). The difference could be traced to the difference in the dosages employed. There may be also individual differences in sensitivity to the drug (Wagner[6]), and there are undoubtedly species differences. No retinal disturbances were seen when the drug was tested in animals prior to its use in humans, and experiments specifically directed toward the production of retinal pigmentary changes in rabbits, dogs, and rats were unsuccessful (Wagner[6]).

We had the opportunity of studying the retinal functions in two of the patients from the series of Goar and Fletcher. This paper is a report on the findings in that study.

Clinical Reports

CASE 1.—A 19-year-old white woman was admitted to the hospital with an acute depression following pregnancy. She had been given chlorpromazine, without benefit, prior to her treatment with NP 207. From June 3, 1955, the patient received NP 207 over a period of six and a half weeks, the total dose being 42,300 mg. She was given as much as 2800 mg. daily for a period of six days. When first seen by one of us (M. C. F.), on July 15, 1955, the patient had no ocular complaints. The vision without correction was 20/30 in each eye, and no abnormality was found in a complete ophthalmologic examination. On July 26,

1955, the patient reported rapid loss of vision during the previous week; the visual acuity was 20/100 in the right eye and 20/200 in the left. The discs were injected, and there was retinal edema. In the far periphery of the retina of the right eye there was fine pigmentation in the lower temporal area. The visual fields showed a general constriction of 10 to 20 degrees in each eye. The ophthalmologic staff of the hospital made at that time a diagnosis of hysterical blindness. On Aug. 2, 1955, there was no change in the appearance of the fundi, but on Aug. 29 both retinas presented definite abnormal pigmentation and a toxic retinitis was diagnosed.

From that period on there was slow and steady recovery over the following six months. The last complete ophthalmologic examination was made on July 25, 1957, at which time the uncorrected vision was 20/60 in each eye. The muscle balance was within normal limits; the eyes were white and quiet, and the media were clear. The fundus examination revealed in both eyes a slight injection of the discs. The blood vessels were normal in size and distribution, but there were numerous small capillaries in the periphery. The most outstanding abnormality was the presence of large sheets of pigment in the retinas. Some abnormal pigment formations resembling spiderwebs had appeared since her previous examination. These somewhat resembled the bone corpuscle formation seen in retinitis pigmentosa. The testing of the visual functions reported in this paper was done in October, 1957.

It is of interest to note that this patient, along with all the other patients treated with NP 207, obtained rapid improvement of her mental symptoms. She has had no recurrence of her depression, despite a second pregnancy.

CASE 2.—A 31-year-old Negro woman was admitted to the hospital with schizophrenia. This was her first attack. She, likewise, had been treated with chlorpromazine, without benefit. Her NP 207 treatment was begun on June 14, 1955, and continued over a period of nine weeks. The total dose given was 45,900 mg. She was first seen by one of us (M. C. F.) on Aug. 29, 1955. At that time she complained of blurring of vision of one week's duration. Inspection revealed a rather staring gaze with exotropia. The pupils were partially dilated but reacted normally. Fixation was poor in both eyes, and the uncorrected visual acuity was 20/200 in each eye. The media were clear. The fundi presented an increased redness of both discs and an amorphous pigmentation throughout the midzone and far periphery of both retinas. There was no involvement of the peripapillary area or of the maculas. On Sept. 7, 1958, the patient had her greatest visual loss, the acuity on the right being reduced to hand movements, on the left to 20/300. During the ensuing months there was a slow recovery of vision. When seen on Feb. 1, 1958, two and a half years after the original illness, the patient had 20/200 vision in each eye and read Jaeger 4 with the right eye and Jaeger 7 with the left.

The studies of the visual functions were performed in March, 1958. At that time it was noted that the discs had good color and that the retinal vessels were normal in size. Immediately surrounding the disc and throughout the entire region of the posterior pole the retina had a grayish, almost slate-like, metallic appearance which formed the background for innumerable fine pigment spots. Here and there small round or slit-like openings appeared in this background which had the red color of choroidal vessels. The macula was occupied by the slate-like color, but the fovea was seen as a reddish round area containing golden-yellowish glistening spots. At the edge of the slate-gray area there was some relatively normal-looking retina, but there were a number of tongue-like protrusions of brownish sheet-like pigment, especially in the upper and temporal region. Temporally and temporally below, the pigment had a spiderweb shape and there and in the lower part of the

retinas resembled somewhat the pigment seen in retinitis pigmentosa.

The patient has not had a recurrence of her schizophrenia and is now working.

In addition to the findings in these two patients we shall refer in this paper for purposes of comparison to a normal subject and a patient with retinitis pigmentosa.

The *normal subject* was a 21-year-old white male student whose vision was 6/4.5 in each eye without correction of his insignificant refractive error. Slit-lamp and ophthalmoscopic examinations revealed no pathologic changes of any kind.

The *patient with retinitis pigmentosa* was a 27-year-old married white social worker who had known for the past 10 years that she had night blindness. With correction for her myopic astigmatism the patient had a vision of 6/6-3 in the right eye and 6/6-1 in the left. The anterior segment showed no abnormalities, and the lenses were clear. She had waxy-appearing discs, thread-like retinal arterioles, and some scattered pigment typical of retinitis pigmentosa, though in moderate quantity, in the midperiphery of both retinas. She had a bilateral hearing loss due to congenital nerve deafness but otherwise was found to have no anomalies in neurologic and general medical examinations.

General Procedure

The NP 207 cases came from Houston to Iowa City and were hospitalized. They were not using any medication and were kept on general diet. All visual tests were done after maximal dilation of the patients' pupils with 2 to 3 drops of a 0.5% cyclopentolate (Cyclogyl) hydrochloride solution. The tests consisted of repeated examinations of the visual fields, the dark adaptation, and the electric responses of the retinas to photic stimulation. Because of the restricted time during which the patients were available, a large number of tests had to be performed in the course of a day, but it was attempted to give the patients adequate rest periods between tests.

The normal observer and the patient with retinitis pigmentosa were examined on an outpatient basis. The tests were not given to these subjects in as concentrated a fashion as they had to be given to the NP 207 patients.

Perimetric Studies

In addition to routine Bjerrum screen tests (1/1200, 3/1200 and 5/1200 test objects) a series of tests with the Goldmann perimeter were conducted. Two object sizes (64 sq. mm. and 1 sq. mm.), two relative intensities (1.00 and 0.96), and three colors (white, red, blue) were employed. The results of the tests were essentially the same for both eyes of the NP 207 patients. To avoid repetition we shall only present the findings on the left eye in Case 2 (Fig. 1).

With the white 64 sq. mm. object and a relative intensity of 1.00 an outer isopter was obtained. When the relative intensity was reduced to 0.096 a peripheral ring, a central island, and some scattered islands of responsive retina were found. With use of the 1 sq. mm. white object and relative intensity 1.00 the peripheral ring disintegrated into a nasal and temporal crescent. Only a minimal central crescent and two peripheral spots remained in the field when the white 1 sq. mm. object of relative intensity of 0.096 was employed as target.

The red test object of 64 sq. mm. and 1.00 relative intensity yielded two peripheral crescents and a central island, whereas only a small paracentral crescentic island was left when the relative intensity was reduced to 0.096. The red object of 1 sq. mm. was not perceived, regardless of the relative intensity.

Using the blue test object, a field could be obtained only with the 64 sq. mm. object at the relative intensity of 1.00. This again gave peripheral crescents and central and peripheral islands.

For purposes of comparison with the results obtained in Case 2, the field charts of the right eye of the patient with retinitis pigmentosa are reproduced in Figure 2.

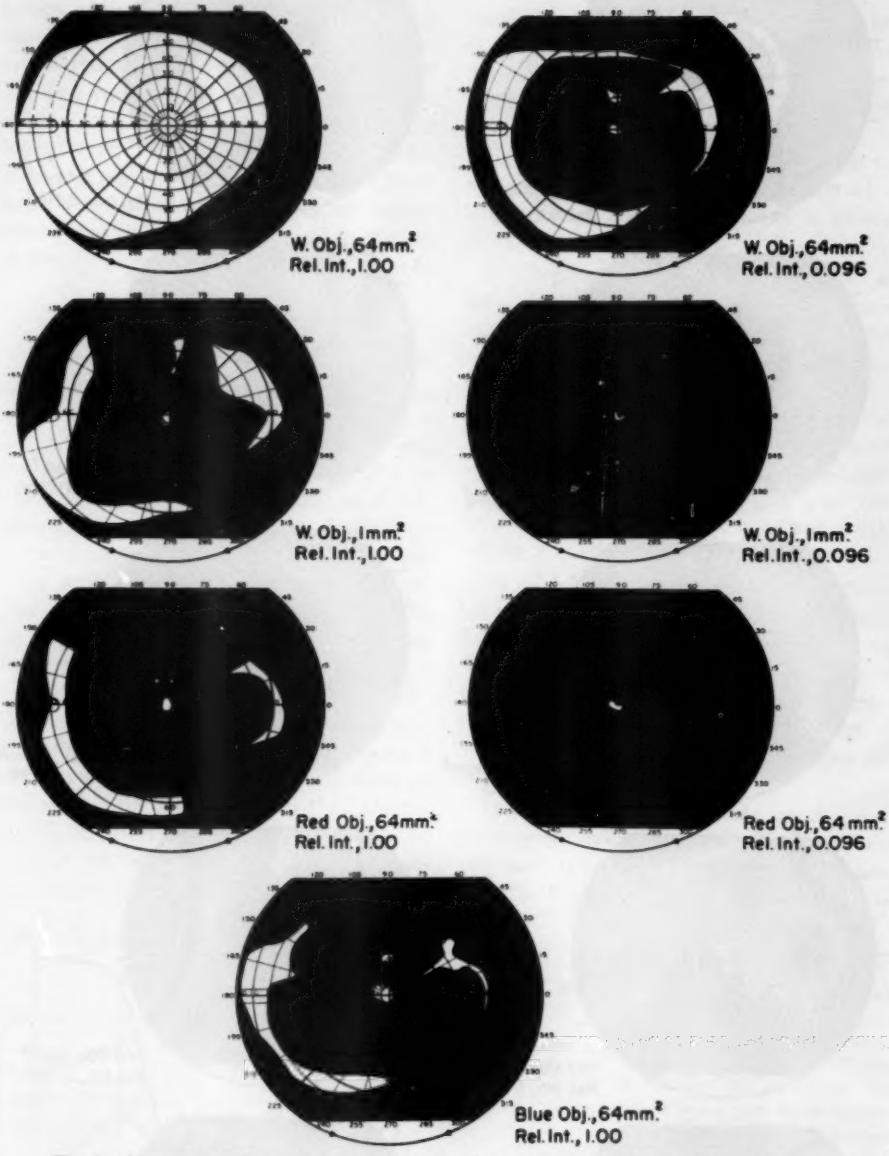

Fig. 1.—Visual field charts of left eye of NP 207 patient, Case 2, obtained with the Goldmann perimeter.

The same objects, relative intensities, and colors were employed in the two patients. It is evident from the charts that the reduction either in size or in relative intensity of the objects resulted in a pronounced concentric restriction of the perimetric fields, which were abnormal even in the outer isopters.

The alterations of the visual fields both in Case 2 and in the patient with retinitis pigmentosa demonstrate a severe affection of the rods as well as of the cones. The af-

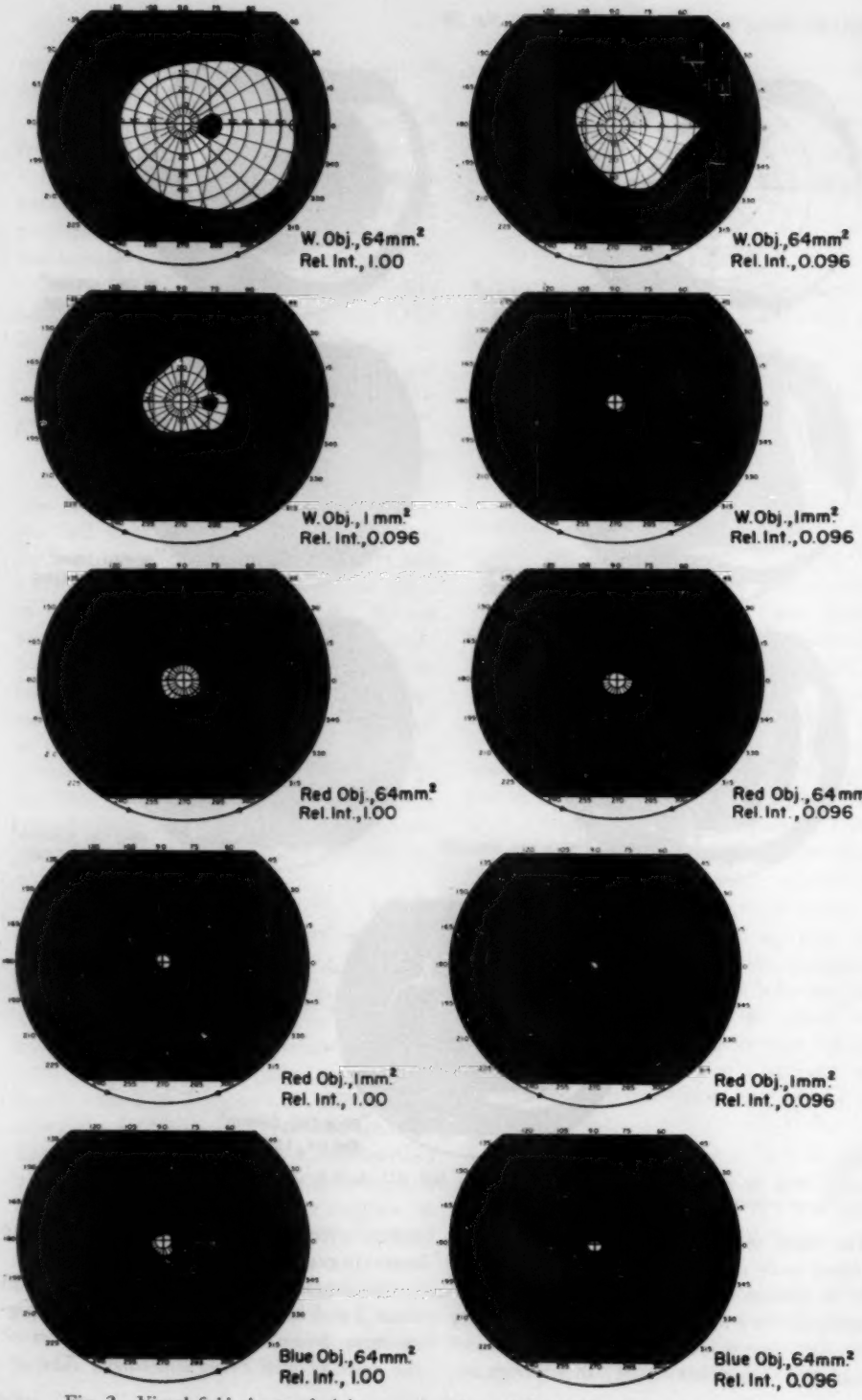

Fig. 2.—Visual field charts of right eye of patient with retinitis pigmentosa, obtained with the Goldmann perimeter. Note that no field could be obtained with the 1 sq. mm. blue object.

fection of the rod function was even greater than that of the cone function, as evidenced by the greater loss of field with blue targets. The two patients differed in their response insofar as there was a uniform loss in the patient with retinitis pigmentosa, whereas in Case 2 the loss was spottier: Peripheral crescents and scattered islands of higher retinal sensitivity were preserved in the sea of deeply depressed retinal responsiveness.

Adaptometric Studies

Perimetric studies are not fully adequate to give a picture of the relative involvement of the rod and cone function in a disease process (Sloan [7]). We have, therefore, subjected the patients to adaptometric tests in which we aimed more especially at a differentiation of these functions. Restriction in the time during which the NP 207 patients were available to us made it impossible to carry out as many different tests as we would have wished. However, each of the results presented here is based on at least three or four repeat examinations. The tests showed a remarkable consistency, in spite of the fact that they represented subjective judgments, which are difficult at best and all the more so when they are required of untrained observers and in the presence of abnormalities of the retinas. One might also be tempted to question the reliability of the NP 207 patients in view of their history. It must be pointed out that these patients proved actually to be most willing and cooperative. The patient with retinitis pigmentosa was exceptionally intelligent and had a keen interest in the study. The normal observer—one of a larger group under study—was a student of superior intelligence.

1. *Technique and Procedure.*—In all dark-adaptation tests the Goldmann-Weekers adaptometer was employed. All patients were tested after the pupils had been maximally dilated with 2 or 3 drops of a 0.5% cyclopentolate solution given at an interval of three minutes. Before every single test the eye, or eyes, were preadapted for a period of five minutes to a brightness level of 3000 lux. The test light was always set for a maximum brightness of 6 lux.

In all patients two groups of tests were performed. The first group aimed at establishing the basic dark-adaptation curve. This was obtained from the two eyes simultaneously and using a uniform ground glass test plate 11 degrees in size, the center of which was located 11 degrees above the fovea.

In the second group of tests, which aimed more specifically at a differentiation of the rod and cone function, certain modifications in the adaptometer were made. The

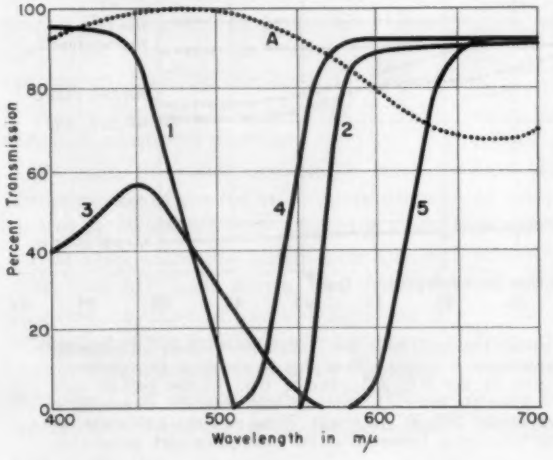

Fig. 3. — Characteristics of filters used in the adaptometric and electroretinographic tests. Curve *1*, Schott BG 12; Curve *2*, Wratten No. *22*. These two filters were used in connection with the Goldmann-Weekers adaptometer. Curves *3*, *4*, and *5*, blue, amber, and red filter used in connection with the Grass Photo-Stimulator. Curve *A*, spectral energy distribution of the gas-discharge tube of the Photo-Stimulator. Note that Curves *3*, *4*, and *5* are not corrected for Curve *A*.

test plate used was either a ground glass plate 5½ degrees in size or else the ground glass was replaced by an orange filter (Wratten 22) or a blue filter (Schott BG 12). The transmission curves of the filters are shown in Figure 3. The test field was fixated centrally by means of a small red light, the intensity of which could be adjusted by a rheostat. Following a suggestion by Gunkel,[8] the light was attached to an arm which was so curved as to correspond to the inner curvature of the bowl of the adaptometer. The position of this arm could be changed in the horizontal meridian by a knob on the outside of the top of the bowl. A rigidly connected pointer indicated on a scale the position of the fixation light in degrees from the center of the test field. In the study of the so-called "retinal profiles" the patient was instructed to turn his eye—but not his head, which was kept fixed—in such a manner as to fixate steadily the fixation light at whatever position it was placed. In this fashion retinal areas whose center was up to 50 degrees nasally or temporally from the fovea could be investigated with ease.

2. *Results.*—(*a*) Basic Dark-Adaptation Curves: In Figure 4 the basic dark-adaptation curves of the normal observer, the patient with retinitis pigmentosa, and the two NP 207 patients are reproduced.

The dark-adaptation curve of the normal observer clearly shows the duplex nature of the dark-adaptation process. In the first five and one-half minutes there is a rapid increase in retinal sensitivity, corresponding to the cone phase of dark adaptation. After this period there occurs a second much slower increase in retinal sensitivity, which represents the rod adaptation. Eventually a rod threshold is attained which indicates in this observer an increase in rod sensitivity of 5×10^5 after 45 minutes of dark adaptation.

The dark-adaptation curve of the patient with retinitis pigmentosa differs greatly from that of the normal observer. It is immediately evident that there is no duplex curve. The retinal sensitivity increases from the beginning of dark adaptation, though nowhere as fast as in the normal observer. After four minutes the sensitivity has increased only 20 times. After the 10th minute there is another very slight downward trend, and at 45 minutes the retinal sensitivity shows a total increase of only about 75 times. This behavior of the dark-adaptation curve of the patient with retinitis pigmentosa clearly establishes that there is

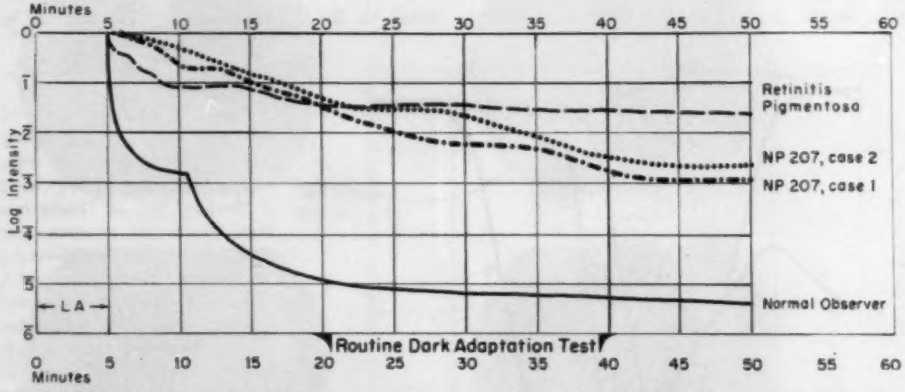

Fig. 4.—Routine dark-adaptation curves obtained with the Goldmann-Weekers adaptometer (field size 11 degrees, located 11 degrees above fovea). Note that in the first 15 minutes of dark adaptation the threshold is higher in the NP 207 patients than in the patient with retinitis pigmentosa, that the curves cross at about 15 minutes, and that the threshold at 45 minutes is 1 to 1.2 log units lower in the NP 207 patients. These patients are worse off than the pigmentosa patient in the first 15 minutes, better off in the subsequent dark adaptation.

considerable damage to the cone system. The rod function is for all practical purposes absent.

The most striking feature of the dark-adaptation curves of the NP 207 patients is the enormous delay of the adaptation process. Adaptation does not set in immediately, but after a lapse of one-half to one minute. By the 10th minute the retinal sensitivity has increased only 9 to 10 times (as compared with an increase of 6×10^4 in the normal observer). In Case 2 the curve is represented virtually by a straight line, up to the 16th minute, after which time it remains on a constant level until around the 25th minute, when another rather considerable drop occurs, with a leveling off around the 40th minute. Except for a short plateau between the fifth and the eighth minute the dark-adaptation curve of Case 1 also approximates a straight line up to the 25th minute. Then another drop occurs with a leveling off at the 40th minute at a somewhat lower threshold than in Case 2.

(*b*) Special Dark-Adaptation Studies: Inspection of the basic dark-adaptation curves would seem to indicate that the damage to the cone system is even severer in the NP 207 patients than in the patient with retinitis pigmentosa. At least it can be said that the cone threshold in these patients reaches that of the pigmentosa patient with a delay of 10 minutes. With regard to rod adaptation the NP 207 patients appear to be considerably better off. However, one can not be sure of these conclusions, since the thresholds, in absolute terms, are high and lie at or above the cone threshold of normal observers.

We have, therefore, attempted a further differentiation of the rod and cone functions by means of special dark-adaptation tests.

(b^1) Dark-adaptation curves with colored lights. Wald[9] and Zeavin and Wald[10] suggested the use of colored lights as a refinement of the technique of adaptometry. Orange light stimulates primarily the cones, and blue light, primarily the rods. When appropriate filters are used, the cone and rod adaptation curves can be separately recorded. This is especially important for the early part of the dark-adaptation curve. When white light is employed as a stimulus, the lower threshold of the cones makes it impossible to detect the threshold of the rods during the period of cone adaptation. When a blue filter is used, the rod thresholds can be traced. Accordingly, when eyes are tested successively with light obtained by the use of orange and blue filters, one finds that in the first few minutes of dark adaptation the cone threshold is lower than that of the rods. At about eight minutes the two curves intersect and the rod threshold becomes increasingly lower than the cone threshold. This is clearly shown in Figure 5 in the curves obtained from our normal observers.

In these tests the observers were fixating centrally the white, orange, or blue discs. Each test was completed from beginning to end with one of the three colors and repeated with a different color on another occasion.

A glance at Figure 5 demonstrates the difference between the normal observer and the patient with retinitis pigmentosa. In the latter patient not only is there a delay in the onset of the curve when the blue filter is used but there is no intersection of the rod curve with the cone curve. The rod threshold is at all times higher than the cone threshold. How severe the damage to the rod mechanism is can be seen from the fact that in this patient the final rod threshold shows that there was an increase in sensitivity of only 80 times, whereas in the normal observer this increase was 10,000-fold. For comparison, the cone threshold of the patient with retinitis pigmentosa indicated a 400-fold increase in sensitivity, whereas it was 6000-fold in the normal observer.

The curves obtained by the same method from the NP 207 patients are especially interesting (Fig. 5). The tremendous delay in the dark-adaptation curve, alluded to before, becomes particularly evident. The rod

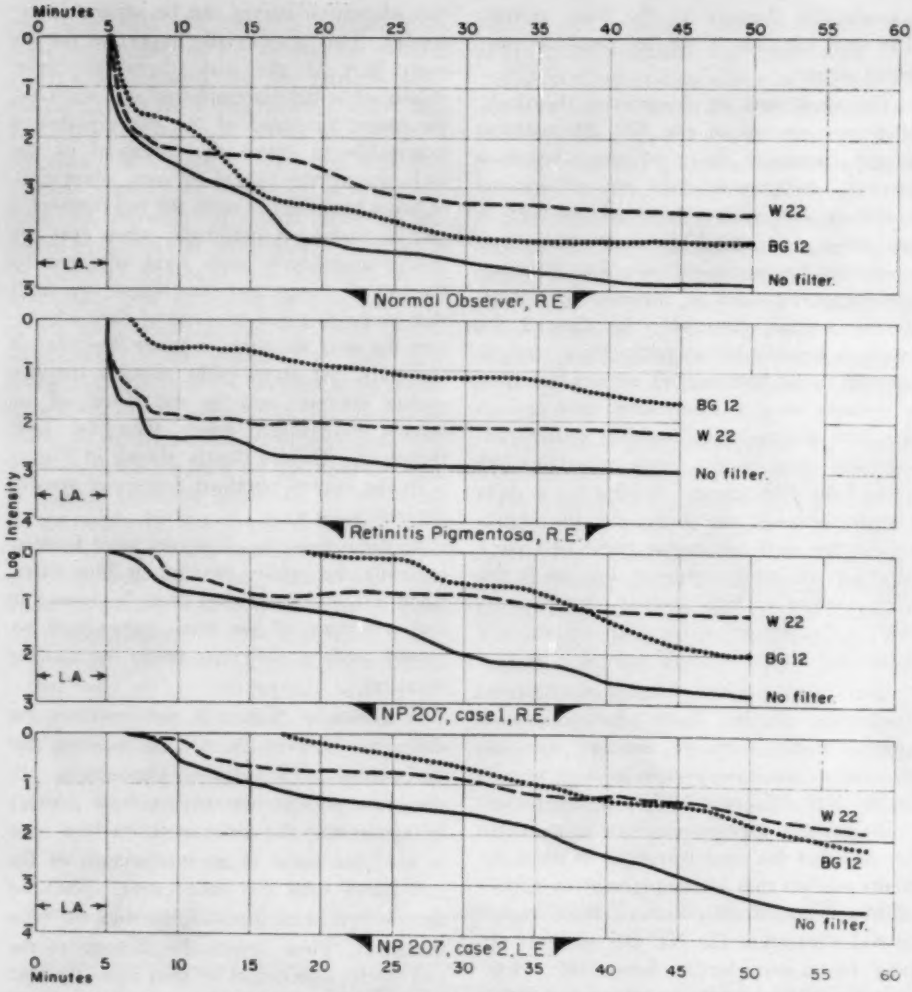

Fig. 5.—Dark-adaptation curves using a centrally fixated field of 5½ degrees. W22, orange filter; BG 12, blue filter. In the normal observer the curve obtained with blue light (rod stimulation) is higher than that obtained with orange light (cone stimulation) in the first seven minutes of dark adaptation, after which the two curves cross and the blue threshold becomes lower than the orange threshold. In the pigmentosa patient the blue (rod) curve never intersects the cone curve. Note in the NP 207 patients the slow cone adaptation (W22) and the enormously delayed rod adaptation (BG 12), but note also that there is eventually (though only after about 35 minutes) a crossing of the curves for blue and orange light.

curve (blue filter, BG 12) does not begin at all for 12 to 14 minutes. Only after this long time interval could a rod threshold be registered. From then on it increased very slowly but rather steadily (especially in Case 2). There was a slight break evident around 30 to 35 minutes of dark adaptation. And it was only at this point that the rod curve crossed the cone curve so that the final thresholds were arranged in the same order as in the normal observer: orange highest, blue next, and finally white. To be sure, these final thresholds are very much higher than in the normal observer, indicating the severe damage which both cone and rod systems had sustained. It is worth

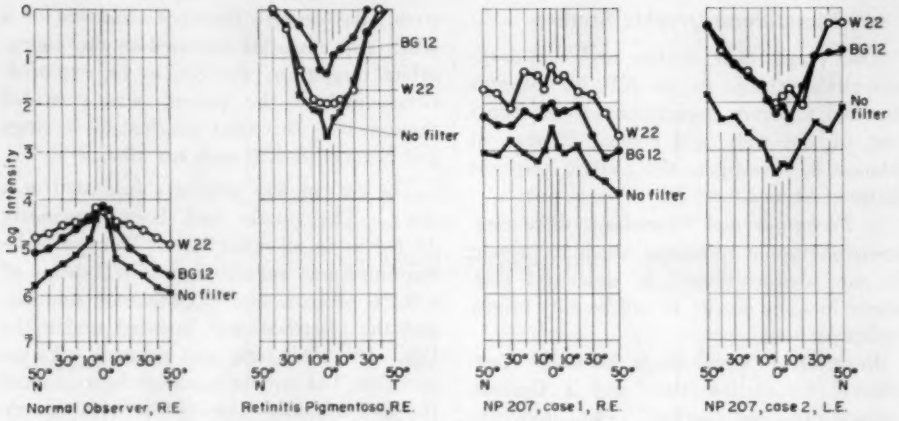

Fig. 6.—Retinal threshold profiles in the horizontal meridian. W22, orange filter; BG 12; blue filter. Note the inversion of the relation of central and peripheral thresholds in the pigmentosa patient and the NP 207 patient, Case 2, in comparison with the normal observer. In the pigmentosa patient the blue rod thresholds could not be obtained at all beyond 20 to 30 degrees, and, in contrast to the normal observer, they are higher than the orange (cone) thresholds.

noting that the blue threshold is somewhat lower in Case 1 than in Case 2. In Case 1 the final orange and blue thresholds are practically identical.

(b^2) Retinal profiles. Sloan [11] and, somewhat later, Mandelbaum [12] introduced a new technique into the testing of dark adaptation by determining the threshold of various retinal areas at specified levels of dark adaptation. These perimetric dark-adaptation curves were aptly termed "retinal profiles" by Zeavin and Wald.[10] The retinal profiles of the horizontal meridian of the normal fully dark-adapted eye (Fig. 6) are characterized by a higher threshold of the fovea than of the periphery, a fact in keeping with the known density of the cone and rod population in different parts of the retina. When the use of orange and blue filters is added, the retinal profiles can be further differentiated with regard to the cone and rod function.

All the authors mentioned have made the interesting observation that in patients with retinitis pigmentosa there was an inversion of the retinal profiles such that the threshold of the central retinal areas was now lower than that of the periphery. This was a consequence of the relatively greater damage to the rods than to the cones. In absolute terms the thresholds of the cones and, especially, those of the rods were, of course, much higher than in normal observers. Figure 6 shows that this held also for our patient with retinitis pigmentosa. In addition, there was the reversal of the orange and blue thresholds, those for blue being higher than those for orange. With blue stimuli no thresholds could be recorded with the method employed by us beyond 20 to 30 degrees to each side of the fovea in the horizontal meridian.

The NP 207 cases presented a different picture. There was a clear-cut inversion of the profile in Case 2, just as in retinitis pigmentosa, but in absolute terms the thresholds were somewhat lower and they were nearly identical for orange and blue. In Case 1 there was no inversion of the profile. In spite of the irregularities of the curve due to local variations in retinal damage, the foveal thresholds were higher than the peripheral ones. The thresholds for orange and blue had the same sequence as in the normal subject, and the peripheral thresholds were somewhat lower than in Case 2.

Electroretinographic Studies

The visual field studies and the dark-adaptation studies in the NP 207 patients revealed a severe impairment of the function of the rods and cones. It was of interest to investigate the electric response of their retinas.

1. *Technique and Procedure.*—The electroretinographic technique used at present in our laboratory will be described elsewhere in some detail. It will be only briefly indicated here.

Recordings were made with a four-channel Grass Inkwriter and a Dumont cathode-ray oscilloscope. The inkwriter channel recording the electroretinogram had a time constant of 0.54 second; the amplification was set at 40 volts per millimeter, and the paper speed during recordings was 60 mm. per second. The oscilloscopic tracings were photographed with a Grass Kymograph Camera. Paper and film tracings were coordinated by means of a Time Lapse Indicator (Arnott [13]). Photic stimulation was produced by the gas-discharge tube of a Grass Photo-Stimulator. The Photo-Stimulator had four intensity steps, designated as I_1, I_4, I_8, and I_{16}. The duration of the light stimulus was about 10μsec. The front of the housing of the gas-discharge tube was located 35 cm. from the patient's eyes and was fitted into the opening of a bowl, the inside of which was painted with white nonglossy paint. Two 100 watt bulbs were so mounted on the bowl as not to shine into the patient's eyes and were used to light-adapt them to the light reflected from the bowl.

The Photo-Stimulator could be activated either manually or automatically every four seconds by means of a Hunter Timer.

Colored stimuli could be produced by the use of filters attached to the front of the housing of the gas-discharge tube. The filters used in this study were red, amber, and blue. Their characteristics are shown in Figure 3.

The Burian-Allen speculum contact lens electrode [14] was employed as active electrode; the inactive electrode consisted of a silver cup electrode fastened to the supra-orbital region of the eye to be explored. Grounding of the patient was provided through two electrodes attached to earrings and clamped one to each ear lobe.

The patient was comfortably seated in a chair. The pupils had been previously dilated as in all other tests. The eye to be examined was anesthetized with 2 drops of a 0.5% proparacaine (Ophthaine) solution, and the electrode was inserted under the lids. The other eye was covered with an occluder. The patient was then light-adapted for five minutes. During the fifth minute of light adaptation flashes of I_{16} were started at a rate of one every four seconds and continued on during the first one and one-half minutes of dark adaptation. In the routine tests these were followed by three or four flashes at specified intervals, each group separated from the other by increasingly larger periods of complete darkness. Different test conditions were employed in special tests. These are indicated in the proper place of this paper.

2. *Results.*—The patient with retinitis pigmentosa gave no electric response to photic stimulation at any time up to 60 minutes of dark adaptation and with stimuli of any intensity or color. This was to be expected. An extinguished electroretinogram is characteristic of retinitis pigmentosa at all stages of development, and this feature may serve as an important differential diagnostic sign between retinitis pigmentosa and pseudo-retinitis-pigmentosa.

In the dark-adaptation tests the NP 207 patients acted in many respects in a way which was similar to the behavior of the patients with retinitis pigmentosa. One might have accordingly anticipated also in these patients an extinguished electroretinogram. However, this proved not to be the case. An electroretinogram could be elicited from either patient, but it was profoundly abnormal.

In Figure 7 six electroretinograms of the right eye of Case 1 are reproduced, one

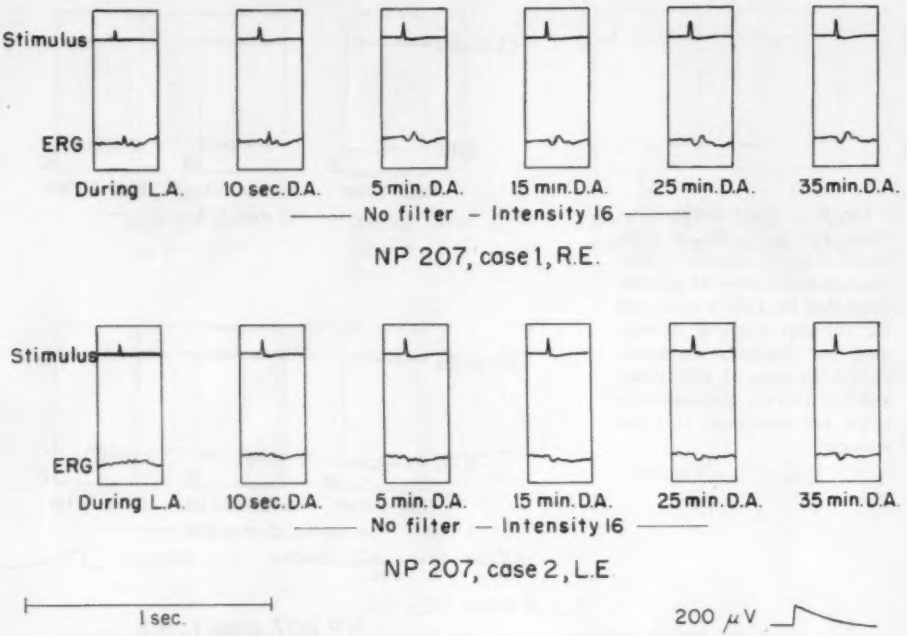

Fig. 7.—Electroretinograms obtained from the NP 207 patients during progressive dark adaptation with the highest available intensity of white light (I_{16}). The ERG's of Case 1 are characterized by a first, sharp positive elevation, followed by a small second positive elevation which increases during dark adaptation. The ERG's of Case 2 show only a negative deflection, but in the later stages of dark adaptation the tracing returns relatively quickly to the baseline, indicating a slight positive activity. Note in both sets of ERG's that the progressive dark adaptation expresses itself in a prolongation of the ERG's.

taken during light adaptation and the other five taken at various stages of dark adaptation, using always white light of maximum intensity (I_{16}). All responses consist of a small, sharp rise, followed by a second very small positive deflection. During dark adaptation a negative deflection (an a-wave) preceding the positive deflections becomes increasingly prominent, and one can eventually begin to distinguish two phases of it (a_1 and a_2). There is also a slight increase in the size of the second positive deflection toward the end of dark adaptation. In addition, there is a marked change in culmination time of the first positive deflection. At first it measures about 25 msec. With the appearance of the a-wave at five minutes of dark adaptation it increases to about 40 msec., and it increases finally to about 50 msec. after 35 minutes of dark adaptation.

We interpret this electroretinogram as representing a more or less pure cone response in the early phases of dark adaptation. The first positive deflection is a so-called x-wave, which is considered to be the result of cone activity. In normal observers it can be isolated only by special means. The x-wave persists in Case 1 throughout the whole period of dark adaptation, but the shape of the electroretinogram is altered by the addition of some rod activity (a_2 and the second positive deflection, the b-wave).

To determine further the contribution of the rod and cone systems in the electroretinogram of this patient, colored stimuli of maximum intensity (I_{16}) were also used. Figure 8 shows the responses to red, amber, and blue stimuli after 15 and 35 minutes of dark adaptation, respectively. After 15 minutes the responses to the three colored stimuli show little difference—they repre-

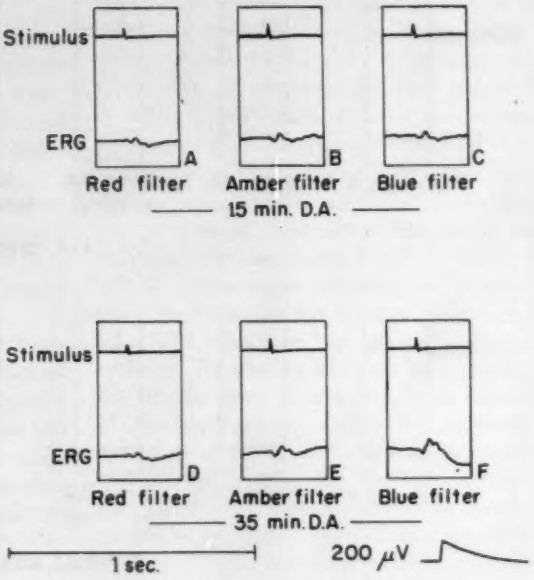

Fig. 8. — Electroretinograms taken with red, amber, and blue filters after 15 minutes of dark adaptation and after 35 minutes. Note that the ERG's at 15, and the red-filter ERG at 35 minutes, are essentially the same; the ERG's obtained with amber and blue filters at 35 minutes are larger and show more of a rod response.

sent more or less pure cone electroretinograms. After 35 minutes, the red stimulus does again not differ in its effectiveness, but the amber light, and more particularly the blue light, which stimulates primarily the rods, demonstrate that the rods are now contributing to the recorded response.

The electroretinograms from the left eye of Case 2 (Fig. 7), taken under exactly the same conditions as the corresponding ones of Case 1, present a different picture. Here there is barely an indication of an electroretinogram during light adaptation or during the initial period of dark adaptation, but after five minutes of dark adaptation a negative deflection appears, which becomes more prominent as dark adaptation proceeds. At the same time, an increase in duration of the electroretinogram (from about 20 msec. to 50 msec.) is evident. This type of response is generally designated as a "negative" electroretinogram. It will be shown elsewhere that this term is not appropriate when the tracing returns more or less immediately to the baseline. For an electroretinogram to be truly "negative" a much slower return of the tracing to the baseline is required.

It was decided to investigate what the response of Case 2 would be if the patient were left for one hour in complete darkness before attempting electroretinography. After five minutes of light adaptation the patient was placed in complete darkness. At 45 minutes the electrode was inserted under the lids in dim red light. This took about one and one-half to two minutes. After this short interruption, complete dark adaptation was continued to the 60th minute. Three or four red, amber, blue, and white flashes, in this order, were then used for stimulation, giving the patient a two-minute interval of complete darkness between each set of flashes.

The results were instructive (Fig. 9). Red light of intensity I_{16} gave a low, slow positive deflection, but no x-wave was evident; amber I_{16}, a shorter, more complex response in which the usual components of the electroretinogram could be discerned.

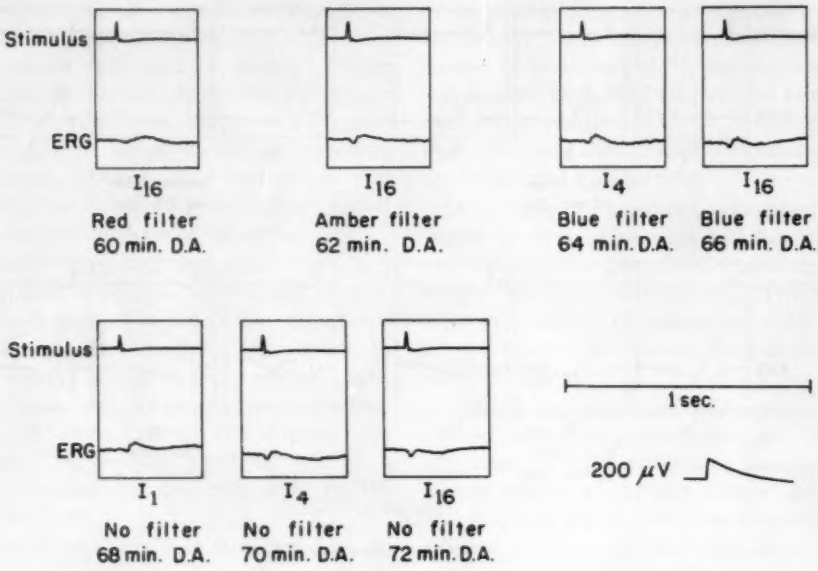

Fig. 9.—Electroretinograms taken after 60 minutes of uninterrupted dark adaptation (NP 207 patient, Case 2, left eye). Note that there is no x-wave with red I_{16} stimulus, and note more particularly the effect of different intensities of white light (no filter) on size and shape of the ERG: With lower intensities the total positive deflection is higher than with higher intensities. With I_{16} the ERG is essentially the same as it was after 35 minutes of dark adaptation (cf. Fig. 8).

Blue at I_4 yielded what may be considered as a pure rod electroretinogram made up of a small a_2-wave and a relatively large b-wave. The a-wave was larger and the elevation of the b-wave above the baseline was lower when blue I_{16} was used as a stimulus. Most interesting was the effect of varying intensities of white stimuli. The lowest intensity (I_1) gave an electroretinogram which was composed of an a-wave and a relatively sizable b-wave (100μvolts). With I_4 the a-wave was greater and the b-wave was slightly smaller, and its elevation above the baseline was lower; with I_{16} measured about 80μvolts. Clearly, with lower intensities we were able to demonthere was no b-wave, but the a_1-wave strate rod activity; with high intensities it was overwhelmed by the cone activity. The electroretinograms obtained with I_{16} at 72 minutes of dark adaptation were indistinguishable from the one obtained at 35 minutes. However, at that earlier period of dark adaptation the use of intensity I_1 did not have the effect of demonstrating rod activity as it did at 72 minutes.

While the electroretinogram of Case 2 showed thus the presence of both cone and rod function, neither could be called in any sense normal. Purely for purposes of comparison, the electroretinograms obtained from the right eye of our normal observer are illustrated in Figure 10. The procedure was comparable to the one used with Case 2. Note that with white stimuli of I_1 the positive deflection measured about 480μvolts; with I_{16}, as much as 600μvolts. Attention is also called to the x- and b-waves, prominent with red I_8 (200 and 280μvolts, respectively) and red I_{16} (265 and 350μvolts, respectively).

In Case 1 the *"central transmission time"* was determined for us by our colleague, Dr. G. K. von Noorden. This time was ob-

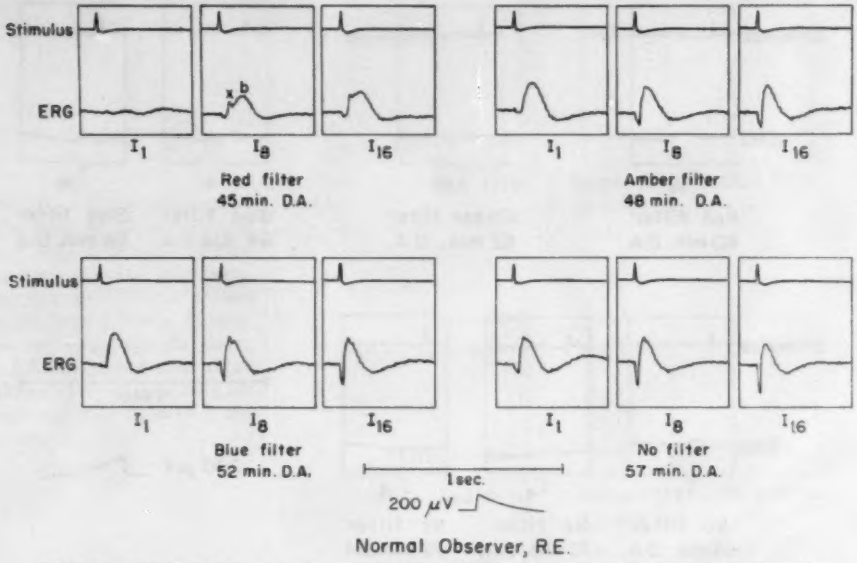

Fig. 10.—Electroretinograms of normal observer taken after 45 minutes of dark adaptation, reproduced for comparison as to size and shape with those from NP 207 patient, Case 2 (Fig. 9).

tained by registering simultaneously retinal responses and cortical responses through the intact skull by driving the cortical rhythm with 7 and 12 cps, after dark-adapting the patient for ten minutes. The "central transmission time" was determined by measuring the time elapsed from the peak of the b-wave to the peak of the corresponding cortical response. While this does not by any means represent the true central transmission time, it is a handy method of comparing normal and abnormal persons. In Case 1 twenty-three measurements of the "central transmission time" were taken stimulating the right eye. The arithmetical mean of these measurements was 120.2 msec. This was 28.1 msec. longer than the normal mean value of 92.1 msec., determined previously on a series of normal subjects. The patient's α-rhythm was 10 cps, with great regularity.

Comment

All the studies made on the NP 207 patients indicated damage to the rod and cone systems of their retinas. We could examine only two patients in whom this damage was very severe. It would have been most helpful to have had for comparison also patients with less severe damage, but unfortunately none could be persuaded to give the time to come to Iowa City.

The relative damage to cone and rod function has been discussed in the section dealing with the adaptometric studies. It remains now to say a few words concerning the electroretinographic investigations.

In retinitis pigmentosa the electroretinogram is extinguished even in the early stages of the disease. This is the experience reported by all electroretinographic laboratories, although one hears of occasional cases in whom a subnormal electroretinogram could be obtained. Certainly in the patient used as an example in this paper no electroretinogram could be recorded, in spite of the fact that her photopic visual acuity was for all practical purposes 6/6 in each eye. In contrast to this, the NP 207 patients did exhibit an electric response, though a very abnormal one. In fact, we were even

able to record an electroretinogram from one NP 207 patient during light adaptation.

It cannot very well be assumed, therefore, that the extinguished electroretinogram in the pigmentosa patient was due solely to the lack of sensitivity of our recording equipment, although it is well known that the electrical threshold is many times higher than the psychophysical threshold.

Noell [15] suggested that, since there is a generalized elimination or marked generalized reduction of rod function in retinitis pigmentosa, the remaining cones might be too widely spread to respond to photic stimulation with an electric reaction which depends upon current flow across the retina. The electrical reactions are, as it were, shunted by the inexcitable or degenerated rods.

Riggs [16] also thinks in terms of a shunting or short-circuiting of the potentials arising in the retina, which consequently cannot be recorded from the remote leads on the eye. This short-circuiting would be brought about by widespread perforations of the retina. Although experiments on rabbits did not fully bear out this hypothesis, Riggs, nevertheless, considered that retinitis pigmentosa caused a specific blocking of the electroretinogram.

Such a specific blocking was not present in our two NP 207 cases, but the abnormalities found in these patients resembled abnormalities of the electroretinogram reported in other conditions.

A small, fast positive deflection preceding the b-wave was first demonstrated by Motokawa and Mita [17] and was later studied more intensively by Adrian.[18,19] It is considered to represent part of the "photopic" response. However, Armington [20] believes the x-wave to be a specific red response rather than a simple photopic response. Schubert and Bornschein,[21] and later Armington and Schwab,[22] demonstrated the absence of the x-wave in protanopia, and Vukovich [23] and François, Verriest, and De Rouck,[24] in achromatopsia. In contradistinction to protanopia, the x-wave was normal in congenital hemeralopia (Schubert and Bornschein [21] and others) and in Oguchi's disease (François et al.[24]), but the b-wave was absent. In normal subjects the b-wave, the scotopic component of the electroretinogram, increases during dark adaptation and can be isolated by blue light.

Our Case 1 presented characteristics similar to those of patients with congenital hemeralopia: An x-wave was present; the b-wave was generally absent. However, after prolonged dark adaptation a trace of a b-wave could be demonstrated with orange and blue stimuli.

Case 2 did not show an x-wave but showed only a negative deflection. After one hour of dark adaptation intense red stimuli elicited a response without x-wave and blue and white stimuli were capable of eliciting a b-wave of moderate amplitude.

Our interpretation of the electroretinogram of the NP 207 Case 1 as consisting essentially of an x-wave may be questioned. However, the amplitude of the first positive elevation in these electroretinograms remained virtually unchanged throughout dark adaptation. Any changes which the electroretinograms of this patient underwent in the later stages of dark adaptation, and through stimulation with blue light, concerned primarily the second positive elevation, traces of which could be discerned in all electroretinograms of this patient. We would suggest that the electroretinograms of the patients with congenital hemeralopia, published by Riggs [16] (especially those of his Subject F, which are similar to those of our Case 1), are also essentially x-wave electroretinograms. Readers interested in the isolation of the x-wave may consult the excellent study of Henkes [25] concerning this subject.

In the absence of pathologic and biochemical evidence one can only speculate about the nature of the damage induced in the retinas by the ingestion of NP 207. Zeavin and Wald [10] have presented an interesting hypothesis to explain the failure of the rod system in retinitis pigmentosa.

They suggest that the cis-isomer of vitamin A, neo-b vitamin A, required for the synthesis of visual pigments, may not be present in the retina in sufficient quantity. What little supply there is is quickly exhausted by the cones, whose specific protein, the cone opsin, unites with enormously greater speed with neo-b retinene (the aldehyde of neo-b vitamin A) than does rod opsin. Thus, no neo-b retinene may remain available to the rods, and their opsin cannot form rhodopsin, with a resulting eventual deterioration of the opsin itself and, with it, of the structure of the outer segments of the rods.

It is unlikely that this explanation applies also to the NP 207 patients. The tremendous delay in dark adaptation, both of the cone and of the rod system, would speak for an inadequate *utilization* rather than for inadequate *supply* of neo-b vitamin A. It is tempting to speculate that in the NP 207 patients there is a primary damage to the opsins rather than to the supply of vitamin A.

The impossibility to produce pigmentary or other retinal changes in experimental animals has been a deterrent to electrophysiologic studies of animals treated with NP 207. One should, nevertheless, consider the carrying out of such studies, since it is well known that abnormal electroretinograms may be found in the presence of normal-appearing fundi and that abnormal electroretinograms may be registered before anatomic changes in the retinas are observed. Should abnormal electroretinograms be encountered in NP 207-treated animals, it might be worth while considering further experimental inquiries into the mechanism of the effect of NP 207 on the retina.

Summary

The retinal function of two patients with retinal pigmentary disturbance due to the therapeutic use of NP 207 was investigated with perimetric, adaptometric, and electroretinographic methods.

The result of these studies showed that these patients had suffered severe damage to both their cone and their rod system.

Their dark adaptation was extremely delayed, and the final thresholds were very high. However, in comparison with a patient with retinitis pigmentosa their rod system was relatively less affected.

In contradistinction to a patient with retinitis pigmentosa, the electroretinogram was not found to be extinguished. In one patient the electroretinogram consisted essentially of an x-wave. In the second patient the electroretinogram showed only a small negative deflection. However, after 60 minutes of uninterrupted dark adaptation an a- and b-wave could be elicited. Intensive red flashes did not produce the appearance of an x-wave.

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A Simplified Method of Stereophotography of the Eye

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In 1954 Donaldson [1] described the latest modification of his camera for medical stereophotography. With his unit, excellent stereophotographs of the external eye, anterior segment, and angle of the anterior chamber could be obtained. Fundamentally, the Donaldson camera is two reflex cameras mounted side by side and connected to a common reflex housing, with use of rhomboid prisms in front to provide a satisfactory interlens distance. There is a mechanism which permits independent adjustment of the interlens distance and parallax displacement. It is well conceived and has produced beautiful pictures. However, the camera is difficult to obtain and is very expensive. For these reasons, it does not fully answer the needs of the average office or eye clinic which desires to improve its photographic records by adding stereoscopy.

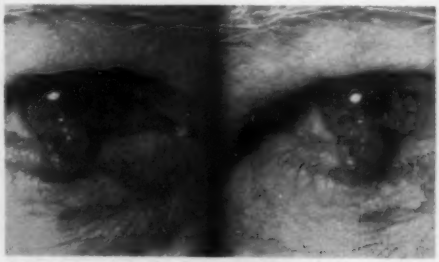
In 1955 Goodstein and his co-workers [2] described a method of taking pictures through a gonioscopic lens, with use of a hand illuminator. The illuminator had a light source for focusing, and a flashbulb in the same optical axis, synchronized with the shutter of a single-lens reflex camera. Magnification was achieved with the use of extension tubes, and a bellows was added for fine focusing. The flashbulbs have since been replaced by an electronic flash light source. This same unit has also been used for photography of the anterior segment.

Recently we were fortunate enough to obtain a Stereoscopic Closeup Lens, manufactured by Zeiss for use on the Kine Exakta camera. This lens consists of two angled prisms placed base to base. When it is mounted in front of the camera lens, the image viewed is split. In this manner, a double image is photographed which, when mounted in a stereoviewer slide mount, can be viewed in a stereoviewer, with excellent stereoscopic detail. For external and anterior segment photography any suitable electronic flash light source can be used. The magnification can be adjusted by the use of extension tubes and accessory lenses as desired. For photographing the anterior chamber angle through a gonioprism, the special illuminator is necessary.

With this technique we have been able to obtain some fine stereophotographs of various types of ophthalmic pathology. Unquestionably, the added dimension of depth makes the pictures more effective. This is particularly true of the anterior chamber and goniophotography.

We have included some of our photographs, just as they came back from the processing laboratory, before mounting (Figs. 1, 2, and 3). In order to see them stereoscopically, hold a piece of cardboard

Fig. 1.—Basal-cell carcinoma (from original color transparency).



Submitted for publication Nov. 25, 1957.

All photographs were taken by the Photographic Department of the New York Eye and Ear Infirmary.

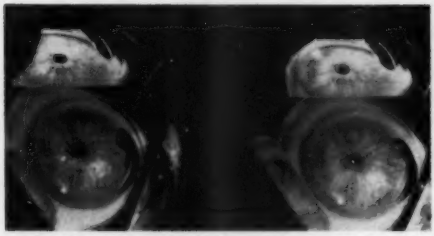


Fig. 2.—Normal angle (from original color transparency).

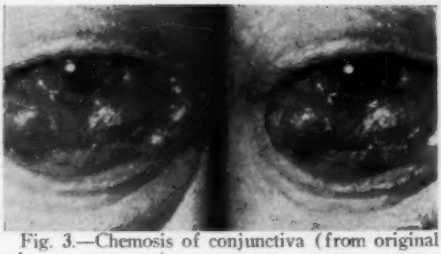


Fig. 3.—Chemosis of conjunctiva (from original color transparency).

about eight inches long in your hand. Place your nose against the other end so that each eye sees one view. By relaxing your convergence you should be able to obtain stereopsis. The stereoscopic image should appear behind the plane of the page.

Comment

The high value of photography in medicine, as a permanent record in the management of individual cases and as a teaching aid, is well known. The use of stereoscopic photography enhances this value further. However, in order for the average ophthalmologist or clinic to make use of it the technique must be rapid and simple and the equipment, relatively inexpensive. We believe that the Stereoscopic Closeup Lens, with use of a single-lens reflex camera, fulfills this need.

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Annual Reviews

Physiological Chemistry of the Eye

A Review of Papers Published During 1957

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Cornea

Maurice [1] proposed a new ingenious theory to explain the transparency of the cornea. It is based on the concept of a lattice structure of the corneal stroma. This lattice structure provides the properties of a diffraction grating, consisting of rows of fibrils arranged in a two-dimensional lattice in such a way that the scattering of the light falling on it is eliminated by interference of the electromagnetic waves scattered from individual fibrils. The author first gives a brief outline of the structure of the corneal stroma based on most recent electron microscopic studies, according to which the stroma consists of parallel lamellae of collagen fibrils which in dry state have a diameter of approximately 25 mμ. The fibrils are imbedded in an adherent ground substance regarded as identical with corneal mucoid. The confirmatory measurements by the author of the refractive index of the stroma of corneas of ox and pig yield values of 1.382 and 1.374, respectively. They were somewhat lower in deeper layers of the stroma than on the surface. The refractive indices of the collagen fibrils and of the interstitial ground substance as well as the volume fractions were determined by extracting the collagen from the stroma of the ox with water or 0.01 NaOH and drying it by heating to constant weight at 105 C. The specific gravity was determined to 1.55. From these data, under the assumption of validity of Gladstone and Dale's law of mixture, the refractive index for the interstitial ground substance was calculated to 1.374, in good agreement with the experimentally determined value. Measurements of the retardation of the fresh isolated cornea of the rabbit, according to a modified method of Stanworth and Naylor (1950-1953), lead to a value of 0.0027 for the total birefringence at the normal thickness of 0.38 mm. The intrinsic birefringence, measured on corneas dried first to constancy in air and then in the dessicator, agreed with that theoretically calculated. From these measurements the values for the refractive indexes of 1.47 and 31 mμ for the fibril diameter were calculated. The author points out that the evidence of double refraction agrees with the picture given by the electron microscope of stromal lamellae made up of hydrated collagen fibrils. It conforms also the concept of fibrils as cylinders of uniform refractive index. Further evidence supporting the electron microscopic evidence of the structure of the stromal lamellae was obtained by diffusion experiments with mammalian hemoglobin and hemoglobin of a mollusk with a uniform molecular weight of 1,630,000. The diffusion constant through a thin corneal strip was found to be about 10 times lower than in free solution, and the diffusion stopped when the cornea was dried to about 50% of its original thickness. The molluscan hemoglobin diffused only when the cornea was swollen in saline to about 150% of its original thickness. Although the molecular diameter of mammalian hemoglobin is much smaller than the calculated distance from the axis of neighboring fibrils, its diffusion

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may be influenced by interaction with the walls of the channels between the fibrils. A calculation of the scattering power of a thus-defined structure of the corneal stroma on the basis of the theory worked out by Schaffer (1909) and checked experimentally by Schaffer and Grossman (1910) shows that under the assumption of no interaction between neighboring lamellae 94% of the incoming nonpolarized light would be scattered and the cornea, therefore, would appear turbid. In reality, the calculation of the light-scattering on the basis of experiments of the author on human cornea, according to a procedure of Langham and Weibar (1954), showed that the total light scattered at all angles except very small ones is less than 1%. The author points out the difficulties encountered by the theory of transparency based on assumptions of uniform refractive indexes of the components of the stroma. To reconcile these theories with the experiments on the scattering, it would be necessary to assume that the corneal fibrils seen in the ultramicroscope are artifacts produced by aggregation of protofibrils with a diameter of no more than 2 mμ. This assumption would be difficult to reconcile with the results of the diffusion experiments and also with the fact that the swollen cornea becomes much more easily turbid than the normal cornea. The explanation of the transparency offered by Cassperson and Engström (1946), which postulates an elimination of reflection on fibril surfaces by an envelope of mucoid with a refraction index tapered from the surface of the fibril to the interstitial fluid, appears to the author not supported by an adequate theoretical treatment. The author shows that if the fibrils of the stromal lamellae are parallel, are equal in diameter, and have their axes disposed in a lattice, the electromagnetic waves scattered from individual fibers will interfere destructively in all directions except in that of the incident beam and, therefore, the tissue will appear transparent. Experiments on the effect of swelling on the clouding of the cornea show that the clouding appears immediately as soon as the volume of the cornea increases. This appears irreconcilable with the arrangement in form of the fibrils in form of a square lattice with the diagonal perpendicular to the corneal surface, as in this case a swelling by 70% will change the square lattice to a stable hexagonal one. The latter form of the lattice appears, therefore, as most probable arrangement in the corneal stroma. The author describes an acoustical analogue to corneal structure.

Dohlman and Anseth[2] made an attempt to determine the swelling pressure of the corneal stroma. They point out that the determination of the swelling pressure by Kinsey and Cogan (1942) and by Pau (1954) led to contradictory results, which may be due partly to differences in the methods and partly to the unpredictable influence of the epithelium and endothelium which were present in preparations of these authors and during the prolonged experiments may have been in the state of necrobiosis. The authors, therefore, scraped off the epithelium and endothelium and Descemet's membrane from the cornea, which was obtained with the surrounding rim of sclera. The material was used about one hour after the animal was killed. The results on the swelling of the corneas were compared with those on scleras Of the latter tissue, 2 cm. square pieces were investigated. The swelling pressure was obtained by determining the osmotic pressure of a dextran solution which, when equilibrated with the tissue enclosed in dialysis tubing, did not produce a significant change in the weight of the tissue. The tissue was enclosed in the tubing in such a way that no air was present inside the bag, and the tubing was flaccid and could not exert any mechanical pressure on the tissue. The experiments were carried out at room temperature at two different pH's, namely, 7.4 and 4.0. The latter corresponded to the over-all isoelectric point of the cornea, and it was assumed that under these conditions no appreciable Donnan effect was present. The osmotic pressure of the dextran solutions was measured with an osmometer

equipped with a glass capillary, and, by presetting the level of the outside fluid, the equilibrium was essentially reached within 72 hours. The piece of dialysis tubing was used as a semipermeable membrane. The weight of the cornea remained constant when dialyzed against a 4.75% solution of dextran. This dextran solution showed an osmotic pressure of about 865 mm. H_2O. Sclera, on the other hand, was in equilibrium with the dextran solution of 2.5%, corresponding to an osmotic pressure of 325 mm. H_2O. The difference between sclera and cornea became insignificant in measurements at pH 4. The authors conclude, therefore, that the higher swelling pressure of the cornea is the expression of a Donnan effect due to the presence of negatively charged nondialyzable mucoid molecules. The value for the swelling pressure of the cornea is only a little smaller than that found by Pau, which used equilibration against another colloid solution, namely, Kollidin 90 (Bayer). This author, however, found for the sclera the same osmotic pressure as for the cornea. The authors believe that this might be due to the fact that in Pau's experiment the cornea was in direct contact with the colloid solution, which could have penetrated the tissue and thus increased the water uptake. As the osmotic pressure of serum was found in control experiments to be equal to that of the sclera, the authors conclude that a specific pumping mechanism must be present in the cornea that removes the water which would enter the cornea and would be attracted by the higher swelling pressure of the corneal stroma.

In a second experimental study the same authors[3] investigated the influence of the intraocular pressure on the hydration of the corneal stroma. The purpose of this investigation was to explain why the removal of the endothelium in vivo leads to a much higher imbibition by the cornea than the removal of the epithelium. To solve this problem, the authors simulated in vitro the conditions under which the cornea finds itself in vivo when the one or the other cellular layer is removed. The experiments were carried out in a pressure chamber which permitted exertion of a hydrostatic pressure by a fluid on either the inner or the outer surface of the cornea. Five corneas were used simultaneously for every experiment. Experiments were carried out at room temperature and 1:1000 thimerosal (Merthiolate) was added to prevent bacterial growth. In a first series of experiments, corneal stroma denuded of the limiting membranes was placed with the anterior side down at the bottom of the pressure chamber, which contained five holes. A pressure of 20 mm. Hg was exerted on the upper posterior surface of the cornea, while the anterior surface was exposed to air. After 16 hours the tissue gained 172% in weight and thereafter leveled off at a gain of about 200%. This set of experiments imitated the conditions in vivo when the endothelium was removed. The conditions after removal of epithelium were imitated by fastening the corneas above the holes at the bottom of the apparatus with the anterior side down and filling the pressure chamber with fluid under an air pressure of 20 mm. Hg, while the anterior side was bathed by an isotonic fluid of zero pressure imitating tear fluid. The pressure by air substituted here for the intraocular pressure. Under these conditions the gained weight did not exceed 125% of the original weight of the cornea. The authors conclude from these experiments that the difference in the effect of the removal of the endothelium and epithelium is due to the fact that in one case the intraocular pressure exerts its influence in the same direction in which the influx of water follows the swelling pressure of the cornea, while after removal of the epithelium the intraocular pressure counteracts the movement of the fluid.

Wortman and Strominger[4] made an attempt to determine the localization of S^{35} incorporated in vitro by adult rabbit and beef corneas. The incubation of whole cornea or pieces of cornea was carried out in Difco TC 199 tissue culture material supplemented with $H_2S^{35}O_4$, at 38 C for 18 hours with addition of 50 units of penicillin

and 6μg. of streptomycin per milliliter of medium. At the end of the incubation the whole mixture was heated for 10 minutes at 100 C; the tissue was washed three times with water, suspended in 0.1 M Na_2SO_4 for three hours at 38 C, and again washed three times with water. This removed inorganic S^{35}. Controls were either heated at 100 C before incubation or stored at −20 C during the incubation period. The radioactivity was either determined by hydrolyzing the tissue with concentrated HCl at 100 C for three hours, precipitating the sulfate with $BaCl_2$ and measuring the radioactivity of the dried precipitate, or on the acid mucopolysaccharide fraction of the cornea prepared according to the procedure of K. Meyer et al. The radioactivity of the isolated polysaccharide fraction was also determined after treatment with hyaluronidase. The mixture of acid mucopolysaccharides thus prepared was then subjected to paper electrophoresis with 0.05 M phosphate buffer at pH 7 on Whatman No. 1 filter paper at a voltage gradient of 20 volts per centimeter for 60 minutes, and the polysaccharides were detected by metachromatic staining with toluidine blue in 50% ethyl alcohol. Radioautography of the electrophoretic strips was also carried out. The paper electrophoresis of the isolated mucopolysaccharide of the cornea, however, showed only one spot with an electrophoretic mobility identical with that of pure keratosulfate. Sclera, on the other hand, showed a spot corresponding to pure chondroitin sulfate. The position of the spot was the same whether it was detected by metachromasia or radioautography. This result was obtained in spite of the fact that pure chondroitin sulfate and keratosulfate could be clearly separated under these conditions. The authors assume that this peculiar behavior of the mucopolysaccharides of the cornea might be due to the fact that the chondroitin sulfate in the cornea has a much higher degree of polymerization than the standards which they used for comparison. Their experiments, therefore, show only that S^{35} is incorporated into the keratosulfate in the cornea and into chondroitin sulfate in the sclera in vitro.

In his Jonas S. Friedenwald memorial lecture, Harris [5] gives a lucid review of his work on the physiological control of corneal hydration. In particular, the results of experiments on the reversible water and cation exchange of the cornea during refrigeration and subsequent return to body temperature are considered. The author weighs arguments for the metabolic concept of mechanisms involved in this exchange as compared with two other possible mechanisms, namely, increase in osmotic pressure due to changes in the ionization of colloidal anions and changes in the bound water. He concludes that the metabolic concepts appear experimentally better supported and regards a water pump rather than a sodium pump as operative in the control of the hydration of the cornea. More recent experiments on the effect of the synthetic sulfate of hyaluronic acid on the hydration and transparency of the cornea are also reported. The material used came from G. D. Searle & Co.; 0.05 cc. of a 10% solution of material was injected into the vitreous, the aqueous, and intracorneally in vivo. In all cases there was a powerful hydration of the cornea which exceeded the maximum hydration obtained under other circumstances. After the intravitreal administration the cornea gained maximally 400% of its normal water value. Even greater was the hydration after injection in the anterior chamber, which started 24 to 48 hours after the second injection and gained a maximum at an earlier period than when injection was made in the vitreous. Heparin, a natural sulfated mucopolysaccharide, under these circumstances had no significant effect. After intracorneal injection hydration was fairly marked 24 hours later. Some hydration was also observed with injections of heparin under these conditions. When the ester, however, was injected in vivo into the eye which was kept after enucleation in a moist chamber in the cold, it not only did not increase the hydration which

takes place under these conditions but decreased the amount of water entering the cornea from the anterior chamber. The secretion of water after transfer to body temperature was also decreased, and so the recovery remained incomplete. The experiments on excised corneas failed to show any effect on the hydration of the cornea. The author concludes, therefore, that the very strongly polar ester replaces some of the polysaccharides of the cornea, probably those at the endothelial and Descemet membranes. This membrane is regarded by Harris as the most effective site of the mechanisms of the regulation of hydration of the cornea. The author doubts that antihyaluronidase activity of the ester has something to do with the increase in hydration in vivo. This is regarded as the effect of an increase in barrier permeability or the increase in pump activity which takes place only slowly. Cataractous changes which eventually develop in the eye into which the substance has been injected could also be due to an initial or part substitution of the ester for the polysaccharide of the lens capsule.

Dohlman and Balazs[6] made an attempt to demonstrate the presence of glycoproteins in the cornea which contained a fucomucopolysaccharide moiety and no hexuronic acid. Cattle corneas, obtained from the slaughter house less than an hour after the animal was killed, were freed from epithelium, endothelium, and Descemet's membrane by scraping. The tissue was frozen with dry ice and cut into 50μ sections. The sections were then extracted with 1000 ml. of distilled water per 100 corneal stromas at 4 C for two days. The extract was centrifuged for 30 minutes at 12000 g and filtered through Whatman filter paper No. 2. The filtrate was dialyzed against distilled water for 24 hours at 4 C, with use of Nojax cellulose casings. The dialyzed filtrate was filtered again and analyzed. The residue of this extraction was then extracted with 10% $CaCl_2$. This latter extraction, however, produced material containing much higher amounts of polyuronides. The water extract was subjected to several fractionation procedures, of which finally the fractionation with ethanol was retained as the only suitable one. In this latter fractionation the water extract was first dialyzed for 16 hours against a 0.1 M acetate buffer of pH 5. A precipitate which was formed during the dialysis was centrifuged off at 25000 g at 2 C, and the ethanol was added dropwise at 0 C. The precipitate was then digested by trypsin and pepsin for three days at 38 C under control of pH and addition of new enzyme every day and was then dialyzed and dried. The undigested precipitate as well as the digest and the supernatant were subjected to analysis of the sugar, sulfur, and nitrogen. The precipitate and the supernatant were then analyzed for hexosamines, which were separated on a resin column (Dowex 50 in H-cycle) and eluted with 1 N HCl. The total hexosamine was determined after hydrolysis for 20 hours at 95 C in 5 N HCl, and determination was carried out by the Blix modification of the Elson-Morgan method. Hexuronic acids were determined by the decarboxylation method of Burkhart, Baur, and Link; the ester sulfate, by the benzidine method, according to Anderson, and hexoses were identified after hydrolysis for 4 or 20 hours in 2 N H_2SO_4 at 95 C and removal of electrolytes on Dowex 50 200-400 mesh by paper chromatography with a pyridine-amyl alcohol-water mixture, according to Werner and Odin (1949). The precipitate of the ethanol showed very little uronic acid and sulfur and 6.6 times as much glucosamine as galactosamine, half of the latter being equivalent to the uronic acid content. The paper chromatography of the sugars indicated the presence of galactose, glucose, mannose, and fucose, but the precipitate gave negative direct Ehrlich reaction and Bial reaction for sialic acid. The authors conclude that the corneal stroma, in addition to acid mucopolysaccharides, contains glycoproteins of the same type as those found in connective tissue by other investigators.

Dohlman[7] studied the fate of various constituents of the corneal graft by transplanting the cornea from a rabbit injected with S^{35} to the cornea of an animal which was not labeled and comparing the rate of decay of the radioactivity in the host with that in the donor. The experiments were carried out on 76 rabbits weighing 2 to 2.8 kg. each, which were divided into four groups. Two-thirds in each group received intravenously 1 mc. of S^{35}. Three days after administration of the isotope, at which time most of the inorganic labeled sulfate was eliminated, the cornea from one eye of the donor was removed and transplanted to an unlabeled animal of a different strain. The other eye was left in the live animal. The four groups of animals were killed 2, 5, 14, and 40 days, respectively, after the operation. The cornea of the one eye of the donor animal as well as the transplanted cornea and the corneal residue of the eye after transplantation, which had been preserved frozen at −15 C, were removed, dried at 90 C over P_2O_5 to constant weight, measured directly for radioactivity with a Geiger-Müller counter, and finally combusted, after which the radioactivity again determined. To determine the fate of cellular elements of the corneal graft, the cornea of donor animals was labeled with P^{32} by injecting subconjunctivally 4 mc. of labeled *o*-phosphate in 0.1 ml. of saline in each eye. Eight days later two corneal transplants were cut out with a 6 mm. trephine from symmetrical places in the same donor cornea, and their radioactivity was measured. One of the two grafts was then transplanted, and the other was kept frozen at −15 C. After 2, 6, or 10 days the recipient rabbits were killed; the grafts were removed and divided into epithelium fraction and endothelium plus Descemet's membrane. The control grafts were treated in the same way. The tissue samples were then digested with papain in phosphate buffer pH 6.7 for two hours at 60 C, which led to complete solution; the solution was precipitated with TCA, and the precipitate was washed and dried and its radioactivity was measured, under the assumption that the self-absorption in the experimental and control graft fractions was the same. The decay of the radioactivity in the TCA-insoluble fraction, which was supposed to contain the nucleic acids, was then computed, under the assumption that the radioactivity in the grafted sample at the moment of grafting was the same as that in the control sample. In the corneal transplants the radioactivity initially decreased at a considerably higher rate than in the normal cornea, and after 14 days it went down to a level only one-third as high as that in the control. There was no correlation between the rate of elimination of S^{35} and the clinical result of the grafting. The elimination of radioactivity from the TCA-soluble and TCA-insoluble fractions of the three corneal layers was determined in the donor animals by enucleating one eye 8 days after intravenous injection of P^{32} and the other 10 days later. The half-time of the elimination was then obtained by extrapolating the line of decay to zero. The elimination of the graft was determined by comparing the radioactivity of the three corneal layers at various time intervals after transplantation, which took place also eight days after labeling. The half-life of the TCA-soluble fraction was 45 days in the epithelium and 13 days in the stroma; that of the insoluble fraction, 18 and 8 days, respectively. The determinations for the endothelium were uncertain. The elimination from the graft of the TCA-insoluble radioactivity was much faster, more than 95%, after six days in the epithelium and 10 days in the stroma. The possibility of an increased turnover rate of the TCA-insoluble compounds in the graft was excluded by a control experiment in which one and five days, respectively, after keratoplasty on the right eye 2 mc. of P^{32} *o*-phosphate was injected intravenously and 24 hours later the radioactivity in the layers in the cornea of the enucleated eye was determined. It showed that the incorporation of the transplanted graft was not higher than in the control cornea. The

author infers from these experiments that there is a net loss of mucoid from the transplanted graft and that the cellular elements of the transplanted grafts are also removed from the transplant and regenerated from the host tissue. The increased incorporation in the stroma surrounding the transplant also indicates a high rate of cell division there, and this is in agreement with histologic findings of an increased number of mitoses in the host cornea during the first two weeks following keratoplasty.

In another series of experiments Dohlman [8] made an attempt to determine whether the increase in the rate of elimination of S^{35} from corneal transplants is due to the elimination of ground substance or to an increased turnover rate. In the latter case the rate of incorporation should be increased above normal. To this end he investigated the incorporation of S^{35} in vitro into corneal grafts 1, 3, 10, 16, 24, and 34 days after the keratoplasty and compared it with that in normal rabbit corneas. Keratoplasty was performed on 84 eyes in 42 rabbits weighing 2 to 3 kg. each. Two excised corneas with the grafts in situ were placed in 20 ml. of Krebs-Ringer bicarbonate solution containing 0.1% glucose and labeled Na_2SO_4 and incubated four hours at 37 C. The normal corneas were treated in the same way. After incubation the corneas were dried at 90 C to constant weight over P_2O_5; they were then combusted, and the radioactivity was measured. The ability of the graft cells to incorporate the isotope into organic material was found to be greatly reduced immediately after surgery and to recover in the following 10-day period. This depression of the S^{35} incorporation was also observed in the cornea of the recipient animal surrounding the graft. The recovery followed the same pattern as that of the graft itself. Autoradiography of corneas after keratoplasty showed a heavy accumulation of the isotope at the wound edges which coincided with the accumulation of fibroblasts. This labeling disappeared completely on the 34th day after the graft had healed, and the fibroblasts disappeared. These experiments show that in the rabbit, elimination of S^{35} from corneal transplants cannot be due to an increase in the turnover, as the uptake of S^{35} postoperatively drops almost to zero and recovers only during the following 10-day period, in which the elimination of the S^{35} from a prelabeled transplant is far progressed.

Boros and Takats [9] investigated the content of acetylcholine in corneal transplants. The authors discuss extensively the chemical, hormonal, and biological factors which influence the viability of the transplants. They believe that only the cellular elements of epithelium, endothelium, and stromal cells are provided to the transplant by the host, while the stromal fibers as well as Descemet's and Bowman's membranes of the transplant survive. Transplantations of cornea were carried out on 30 rabbits, the size of the transplant being 5 mm. in diameter. The transplant was fixed in its position by the pressure of a celluloid disc, the curvature of which corresponded exactly to that of the cornea. The disc was fastened at four spots and removed after three to four days. At various time intervals after transplantation the corneal transplants were removed and weighed, and the determination of acetylcholine in them was carried out with use of frog hearts as test. For the test the transplant was homogenized, after being weighed, in a bicarbonate-free Ringer solution containing saline; the ratio of fluid to cornea was 50,000:1. The homogenate was deproteinized by heating for 10 minutes at 60 C and then centrifuged, and the supernatant was used for the determination. The intact cornea before transplantation showed a content of 13μg. to 18μg. per gram of acetylcholine. After transplantation the acetylcholine content was 0. It went up continuously in the corneal transplants which remained clear until 20 weeks after transplantation and reached a level of 8.3μg. per gram. The wound edge of the host in these corneas is lower in acetylcholine than the transplant itself. In

corneal transplants, however, which lost their transparency the amount of acetylcholine was only one-third to one-fifth of that of the corneas which remained clear. In these cases the wound edge of the host showed higher values than the transplant itself. The authors point out that these changes in the content of acetylcholine are independent of the nerve supply of the transplant, but they believe that it may be related in some way to the ability of the transplant to survive without losing its transparency.

Münich [10] reports extensive investigations on the content in calf and rabbit corneas of various phosphate fractions as well as the rate of exchange of P^{32} injected subconjunctivally with the corneal phosphate fractions. The experimental set-up unfortunately is very often such that it is difficult to draw any conclusions concerning the metabolic significance of changes which were found by the author. The corneas of calves were obtained from slaughter houses. The effects of temperature, pH, and various metabolic inhibitors on the distribution of phosphorus among four fractions, namely (1) inorganic phosphate, (2) the difficult hydrolyzable phosphate, (3) ATP, and (4) ADP, were determined. This was done by incubating half of the cornea for 60 minutes at 37 C in a nutrient medium used by Merriam and Kinsey for lens cultures. One-half of the cornea was incubated under experimental conditions; the other under standard conditions, as control. One part of the experimental and control cornea was used for the determination of the total phosphorus, while another was precipitated with TCA and the supernatant used for the fractionation of the acid-soluble phosphorus. This fractionation was carried out by paper chromatography, and the above-mentioned four fractions, detected on the chromatograms by the photo print procedure of Martin and Smith and the procedure of Hanes and Isherwood, were cut out and, after washing, were used for quantitative P determinations according to Berenblum and Chain. Experiments on the incorporation of P^{32} were carried out on rabbit corneas. The animals were injected in one eye with a solution of labeled *o*-phosphate, the radioactivity of which corresponded to 4×10^6 counts per minute in milliliters. The phosphate fractions were obtained in the same way as above, and the measurements for radioactivity were carried out on solutions of the ashed materials. The distribution of the phosphate fractions in calf corneas was first determined on a series of duplicate samples of halves of the same calf corneas to determine the standard deviation between the two halves. It must be noted that these distributions are those in incubated corneas and that no determinations were done to determine the original distributions before incubation. The data also are given only for the percentual distribution among the four fractions, and no absolute values for any of the fractions or the whole were determined. The authors also point out that the recovery of the individual fractions in their method was not 100%. The ratios between the individual fractions, therefore, have only a comparative value as far as differences between experimental samples and controls are concerned. The main weakness of this investigation is that no differentiation between the part of the epithelium and the endothelium, on the one hand, and the stroma, on the other hand, was carried out. In only one experiment the total cornea and the cornea after removal of the epithelium were compared. In these experiments, as expected, the ratio of ATP and ADP to other phosphate fractions was strongly increased. Incubation at 20 C showed a very slight increase in Fraction I at the expense of ATP, as compared with controls incubated at 37 C. At 85 C, on the other hand, all ester fractions appeared dephosphorylated to a large extent, as could be expected. The identical sense in the direction of changes by lower and higher temperature does not appear surprising, as the identical changes may result from very different shifts in the ratio of numerous metabolic processes in such a complex system. Shifts of pH be-

tween 7.2 and 8.0 were of no influence. At pH 10 Fractions II and I increased at the expense of Fractions III and IV, while at pH below 6, only Fraction I increased at the expense of all others. KCN 1/1000 M led to a significant decrease in ester Fractions II and III, but unfortunately no significant corresponding increase in inorganic phosphate appeared, which is difficult to understand. Use of 1:1000 M NaF caused a shift from Fraction III to Fraction II, which might indicate that glycogen and glucose were phosphorylated but not glycolized. Monoiodoacetic acid 1:1000 M yielded a picture similar to that of sodium fluoride except that, in addition to the shift from Fractions III and IV to Fraction II, there was also a partial dephosphorylation of these two fractions to inorganic phosphate. Phlorhizin 1/50 M decreased significantly only Fraction III. Urethan in concentrations in which it causes swelling and clouding of the cornea induced dephosphorylation of all ester fractions. Dinitrophenol 1.5×10^{-3} M had no significant effect. The same is true of ammonium nitrate, which was shown to increase phosphorylations in yeast. Colchicine 1:10,000 had no significant effect. The experiments on rabbit eyes were mainly concerned with the effect of inflammatory agents on the ratio of the incorporation in the four phosphate fractions of the cornea. Control experiments carried out with two corresponding halves of the same cornea showed no significant differences between the two halves, neither in the composition of the acid-soluble phosphate nor in the rate of incorporation between the different fractions. Bee venom keratitis produced with 0.1 mm. of a 1:1000 solution 24 hours after injection into the anterior chamber showed a significant shift from Fraction III to Fraction II and a shift in the degree of incorporation from Fractions III and IV into two other fractions. Keratitis anaphylactica, which appeared either 11 to 12 days after injection of 0.05 ml. of horse serum into the cornea or immediately after intracorneal reinjection 14 days after the first injection, produced an identical shift from the ester fractions to inorganic phosphate, except that the changes in Fraction II were not significant. Finally, keratitis dendritica, experimentally produced by infection of one eye of the rabbit with the corneal epithelium scraped from the eye of patients with this disease, again shifted the phosphorus as well as its rate of incorporation from ester fractions to inorganic phosphate. This influence of inflammatory processes can be interpreted, according to the author, as due to a loosening of the structure of the cornea with subsequent release of normally restrained enzymatic processes.

Kinoshita [11] made an important contribution to our knowledge of the metabolism of the cornea by demonstrating the stimulating effect of pyruvate on the direct oxidative pathway of glucose in the cornea. These experiments were stimulated by previous findings of Hermann and Hickman (1948) that upon incubation of corneal epithelium with pyruvate the increase in lactic acid corresponds only to one-half the amount of pyruvate which disappears. The experiments were carried out on fresh cattle eyes, and the corneal epithelium was scraped off with a scalpel. The epithelium was then incubated in an atmosphere of 95% N_2 and 5% CO_2. When the incubation had taken place in presence of pyruvate which was labeled with C^{14} on carbon 1, all the radioactivity of the pyruvate which had disappeared reappeared in form of lactate. The fact that by chemical measurements the increase in lactate corresponded only to one-half the amount of pyruvate which disappeared was shown to be due to the suppression of the endogenous lactate formation in the cornea by pyruvate. This could be demonstrated by adding glucose labeled on carbon 1 and unlabeled pyruvate and measuring the amount of labeling in the lactate form. Only half as much labeling appeared in presence of pyruvate than in its absence. That the direct oxidative pathway is stimulated by addition of pyruvate was shown by adding unlabeled pyruvate and labeled 1-C^{14} glucose. Under these

conditions $C^{14}O_2$ increased eightfold. Addition of 2-C^{14} glucose but not that of 6-C^{14} also increased $C^{14}O_2$. This effect of pyruvate is explained by assuming that the rate of oxidation of TPNH to TPN is the rate limiting reaction in the oxidation of glucose-6-phosphate in the cornea. The lactic acid dehydrogenase of the cornea is assumed to be able to use TPN as well as DPN as its coenzyme. This ability was demonstrated by incubating pyruvate with the dialyzed homogenates of corneal epithelium and measuring spectrophometrically the rate of oxidation of added TPNH. The rate of this oxidation was found to be only one-tenth or one-twelfth of the oxidation of DPNH under identical conditions. It was also possible for the author to demonstrate directly the oxidation of glucose in a dialyzed homogenate of corneal epithelium when TPN as well as pyruvate was added. The author points out that the ability of the corneal lactic acid dehydrogenase to utilize TPN as well as DPN acts like a pyridine nucleotide transhydrogenase in the soluble cytoplasm.

Kuwabara and Cogan [12] continued their studies on lipogenesis in the cornea. In a series of experiments the specificity of oleates as substrate was tested. Three types of experiments were carried out: In one, the substrate was injected into corneas of rabbits in vivo; in the second, corneal buttons after injection were cut out and incubated in plasma, in serum, or intraperitoneally; in the third, corneal buttons were incubated in serum to which substrate was added. Oleic acid and sodium oleate (0.05 cc. of 10% emulsion in saline) produced progressive inflammatory reactions and local necrosis, and fat droplets began to appear in the corneal cells (epithelium, stroma, or endothelium) 12 hours after injection and increased during several days. This lipogenesis was particularly clear when the corneal buttons after injection were cut out, as it was not masked by cellular invasion from the environment. Incubation in serum to which oleic acid or oleate was added was even more effective than the injection experiments. Lipogenesis was here seen as soon as after six hours. This enhancement is apparently due to serum, as incubation of injected corneal buttons in serum to which no substrate was added also sped up lipogenesis. Methyl-oleate and an oleate ester of polysorbate 80 U. S. P. (Sorbital; Tween 80) also produced lipogenesis and necrosis. The latter, however, showed no results after incubation of nonpretreated corneal buttons. No lipogenesis was produced by other fatty acids or their salts or esters, except purified linolenic acid, which, however, still may have been contaminated by traces of oleic acid. Lecithin produced oleic acid reactions, probably owing to liberation of this acid. Fat extracted with ether from rabbit omentum produced sudanophilic intercellular globules in the cells, but the reaction was weak and appeared only much later, after one week, the fat infiltration remaining mainly extracellular. Olive oil was also ineffective, apparently owing to the inability of the cornea to split off oleic acid. Three tissues, namely, blood, liver, and brain, were also tested either in form of implants of pieces of tissue or emulsions, or in form of extracts with organic solvents. Whole rabbit blood or washed red blood cells caused fat infiltration in adjacent fibrocytes, but only after one week and without accompanying inflammation or necrosis. The rabbit blood was ineffective, but human plasma produced some fat formation. Incubation in serum showed some lipogenesis which depended upon the fat content of the serum. Heparinization in vivo increased and enhanced this effect, and serum of xanthomatose patients showed increased lipogenesis only after heparinization. Corneal tissue was completely ineffective, which excludes the possibility that injury to corneal tissue is a factor in lipogenesis. Injections of whole liver produced lipogenesis in the first few days but without inflammation reactions. Incubation in serum to which liver was added was ineffective. Extracts of liver were also effective, as well as the ether-extracted residue. The latter, however,

disappeared after chloroform and methanol extractions. Brain tissue behaved almost exactly like liver. The authors conclude that these results may have bearing on the problem of so-called fat degeneration insofar as they make probable that fat degeneration is the consequence of lipogenesis from oleic acid or products containing it as a constituent and not the result of fat ingestion or liberation of masked fat.

In another series of experiments Cogan and Kuwabara [13] investigated the effect of various physical, metabolic, and biological factors on lipogenesis in the cornea and compared the lipogenesis in corneal tissue with that in numerous other tissues. Lipogenesis appeared in all forms of corneal cells when corneal buttons were incubated in serum to which substrate was added. Sometimes epithelial cells showed fat globules when substrate was injected into the cornea. These globules persisted in epithelial cells even after the disappearance of the fat globules in stromal cells. Also, separated epithelium showed lipogenesis. The fat globules appeared only in the cytoplasm, not in the nucleus. Corneas of human as well as of beef, cat, rat, guinea pig, and rabbit were equally suitable for this type of experiment. Lipogenesis was produced in the pH range between 6 and 8. It was suppressed at pH 5 and above 8.5. It was also suppressed by lowering the temperature to 4 to 5 C or increasing it to 55 C. It was more abundant at 37 C than at room temperature after equal time of incubation. Heating of corneal buttons to 60 C for five minutes abolished lipogenesis; potassium cyanide at 0.02 M had the same effect. Dinitrophenol inhibited lipogenesis only partially even at 0.0025 M. Nitrogen atmosphere diminished but did not abolish lipogenesis. It was fully preserved by storage in the refrigerator for not more than five days. It was completely abolished after 11 days. In a single experiment, corneal buttons incubated in chicken embryo extract for two weeks showed full lipogenesis. Soaking for 4 days in Tyrode solution at room temperature did not effect lipogenesis, but only 30 minutes' incubation in distilled water destroyed it completely. Irradiation by means of strontium β-rays (15000 rep) did not reduce capacity for lipogenesis. Mechanical injury, even slight, like pressure from forceps or from a knife blade, resulted in complete disappearance. Comparable experiments with other tissues showed that peripheral nerves, nerve rods, thyroid glands, and polymorphonuclear cells had no capacity for lipogenesis. The other tissues could be grouped on the basis for their capacity for lipogenesis in two types: One resembled the cornea in showing lipogenesis dependent on the addition of oleate. Some membranes, scleral connective tissue, and aorta belong to this group. On the other hand, all forms of glandular tissue, muscle, colloid plexus, and granulation tissue showed another type of lipogenesis, best represented by the liver tissue, in which the fat globules appeared only in the exposed margins of the tissue, apparently a function of oxygen availability, but they did not require addition of oleate.

Uzman and Jakus [14] made a histochemical and electron microscopic study of the Kayser-Fleischer ring and copper deposits in the stroma of cornea obtained from two patients with hepatolenticular degeneration. The copper deposits in the stroma were soluble in formaldehyde, dilute mineral acids, and Versene, and were insoluble in ethanol and other organic solvents. The authors, therefore, regard them as deposits of copper salts. The copper detectable within the structures designated as Kayser-Fleischer rings had completely different properties: They were insoluble in formaldehyde but highly soluble in organic solvents and resistant to treatment by 12% HCl. They resemble, therefore, copper chelates. The fact that after treatment with formaldehyde the solubility in organic solvents was lost indicates that the organic constituents of the chelate are at least partly of protein nature and are changed by formaldehyde in such a way that the solubility in organic solvents is abolished. The electron microscopic data on the Kayser-

Fleischer ring are in agreement with the histochemical findings. The strongly varying and characteristic colors of the Kayser-Fleischer ring located in the Descemet membrane are, according to the authors, not due to the color of the chelate but are light-interference phenomena. The formation of multiple rings is considered analogous to the Liesegang ring formations produced when certain salts diffuse from a liquid phase into a gel and combine with a constituent of the gel to insoluble substances. The rings, therefore, may be due to a penetration of copper from the anterior chamber into the Descemet membrane. The accumulation of copper in the cornea in the hepatolenticular degeneration may be due to specific affinities of certain constituents of the corneal stroma and Descemet membrane to copper.

Cogan and Henneman [15] described a case of generalized calcification of the cornea associated with hypercalcemia. The turbidity was completely reversible after normalization of the calcium level in blood.

Matumoto [16] made some observations on the metabolism of the cornea under the influence of chemical agents inducing vascularization. Riboflavin deficiency and alloxan injections decreased O_2 consumption and aerobic glycolysis in rabbit corneas during the first three to five days. Later, as soon as the vascularization started, there was an increase.

Trizcinska-Dabrowska [17] investigated histochemically the distribution of glycogen in the cornea under various conditions. Glycogen is normally present only in the epithelium, particularly in basal layers. It was investigated in human cornea after keratoplasty and enucleation and in rabbit corneae after experimental injection of glucose. Glycogen appears when there is a considerable cellular production. In pathological conditions with extensive degeneration it disappears.

Krwawicz and Szwarc [18] investigated the distribution of glycogen in cornea keratoplasty.

Szwarc [19] investigated by histochemical methods the distribution of activity of phosphatases during the formation of postoperational scars in the cornea. Whereas the enzymes were present only in the epithelium under normal conditions, they appeared in the substantia propria near the scar after injury.

Morone [20] investigated the histochemistry of certain enzymes in lyophilized corneae. He found that during storage alkaline phosphatase disappeared but succinic dehydrogenase and cytochrome oxidase remained unchanged.

Taoka [21] investigated the effect of heavy metals, of temperature, and of elimination of epithelium on the permeability of the cornea. $ZnSO_4$ increased, $AgNO_3$ decreased, and $CuSO_4$ left unchanged electrical resistance. The latter showed a moderate change between 45 and 55 C and a marked change at 60 C, at which temperature the cornea behaved like a free-passage membrane. After two hours at 7.5 C, the resistance decreased by 13%; at 5 C, by 27%. Damage to endothelium decreased electrical resistance by 20%.

Takaku [22] separated from the cornea by ultracentrifugation and zone electrophoresis four different proteins and three polysaccharides.

Esposito [23] found that avitaminosis E decreases the anaerobic glycolysis of the cornea by 50% and does not affect O_2 consumption.

Aqueous Humor

Block and associates [24] found in normal aqueous the concentration of γ-globulin lower and that of albumin higher than that in serum. In secondary aqueous all ratios were equal to those in serum. Cortisone decreased the proteins in secondary aqueous from 2.6% to 1.9% without changing the ratios.

Funamoto [25] found a delay by acetazolamide (Diamox) in the restoration of ocular tension after compression.

Twenty-six reviews on the chemistry and serology of proteins of the aqueous, elec-

trolytes, amino acids and enzymes, and reducing substances and on the dynamics of the aqueous formation and the effect of acetazolamide on this formation were reported in the symposium on the aqueous in Munich on May 14 and 15, 1956. Some of the papers also report results of more recent investigations.

Munoz and Sampaolesi [26] and Remky [27] report micromethods for the determination of proteins in the aqueous.

D'Ermo [28] reports a new technique of electrophoretic fractionation of aqueous proteins which requires only 0.2 to 0.3 ml. of material.

Appelmans and Michiels [29] found increased protein concentration in the aqueous of rabbits after administration of 125 to 375 mg. of acetazolamide orally or 20 mg. per kilogram intravenously or 1.5 gm. sulfanilamide orally.

Cagianut [30] determined Na in serum and in aqueous of man and found 176.4±4.3 and 162.9±4.3 mEq. per kilogram of H_2O, respectively. The latter was depressed in inflammatory lesions.

Steiger [31] determined by two-dimensional paper chromatography the amino acids in human aqueous of the anterior chamber. It differed from plasma and lens proteins as far as glutamic acid and lysine are concerned.

Heinzen and Urech [32] report that the time necessary for the Na^{24} and heavy water injected intraperitoneally into rabbits to reach 50% of the plasma level in the aqueous is not altered by 500 mg. of acetazolamide given intravenously 90 minutes before the experiment.

Berggren [33] examined the distribution of phosphatases, esterases, and succinic dehydrogenase in the corneal epithelium and endothelium, the ciliary epithelium, and the trabecular meshwork by histochemical methods in man, chicken, rabbit, and golden ide (Idus idus). The endothelial cells of the meshwork showed the same range of activity as the corneal endothelium. This suggests that these cells regulate the hydration of the trabecular meshwork in the same way as corneal endothelium regulates that of the cornea.

Green and associates [34] continued their investigations on the corticosteroids in the aqueous humor of the rabbit eye. They subjected chloroform extracts of the aqueous to paper chromatography with toluene-propylene-glycol as solvent and separated hydrocorticosterone from another still unidentified steroid. This substance had a much higher R_f in the solvent and was faintly positive to the NaOH blue tetrazolium test. It was extracted with methanol from the paper after the first chromatography and rechromatogrammed with formamide-saturated cyclohexane-benzene, and again the spot was detected by the blue tetrazolium test, extracted with methanol. The chromatographic properties of this substance suggest that it is a highly nonpolar corticosteroid and that it does not appear to possess a Δ^4-3-keto group or an α, β-unsaturation. Its quantity was estimated to 0.5μg. to 1μg. in 0.7 cc. of aqueous.

Friedman and associates [35] determined the rate of turnover of water of the anterior aqueous humor in rabbits by measuring the exchange of tritiated water between blood plasma and aqueous. The technique of the assay is described in detail. The rate of turnover was estimated between 5.2% per minute and 15.6% per minute, which is in general agreement with values previously reported in which the exchange between blood and aqueous was measured. The authors discuss also several sources of error which effect the accuracy of determinations of this exchange, particularly the influence of anesthesia, the difference between the concentrations of the isotopes in the arterial blood and in capillaries, the speed at which the isotope is distributed across the anterior chamber, and, finally, the role of the establishment of equilibrium through diffusional exchange of water between the various parts of the eye.

Langham and Lee [36] investigated the effect of acetazolamide under normal conditions

and after nephrectomy and administration of ammonium chloride on the composition of the aqueous, the rate of formation of the aqueous, and intraocular pressure. In the first series of experiments the influence of acetazolamide on the pH and the concentration of bicarbonate in aqueous and arterial blood were compared. In the second, the time of appearance of fluorescein under the influence of acetazolamide was studied and its effects on the maximum ascorbic acid level in the aqueous after injection into the blood stream were determined. In a third series of experiments changes in the intraocular pressure under the influence of acetazolamide and ammonium chloride during the period leading to a new steady state were measured. The immediate purpose of this investigation was to resolve the contradictions concerning changes in the chemistry of the aqueous between the findings of Green and associates and those of Becker. To this end, certain methodical drawbacks in previous experiments were eliminated by the authors. Adult rabbits of both sexes were used; the aqueous was tested for the presence of protein by precipitation with TCA, and samples displaying more than the normal clouding were rejected. pH determinations were carried out on the whole blood instead of on plasma at room temperature and were recalculated for the body temperature after a determination of the temperature coefficients for blood and aqueous. The total CO_2 of the blood and aqueous was determined by the manometric technique of van Slyke. The concentration of bicarbonate was calculated from these values and the pH by the Henderson-Hasselbach equation. The intraocular pressure was measured according to Greaves and Perkins (1952) in anesthetized animals and by tonometry in conscious animals. The concentrations of bicarbonate and hydroxyl ions in the aqueous exceeded those in the arterial plasma calculated from the blood values from the nomograms of van Slyke and Sendroy. The CO_2 tension in the aqueous was found approximately equal to that in blood. Fifty minutes after injection of acetazolamide the pH of the blood was significantly below that in controls, but the concentration of bicarbonate remained unchanged. Consequently, pCO_2 in the blood increased significantly. In the aqueous the pH was also lowered but remained significantly above that of blood. The bicarbonate was lower than in the controls and closely approached that in the plasma. Thus the excess concentration of bicarbonate in the aqueous dropped nearly to zero within 50 minutes after acetazolamide administration. Two and one-half hours after injection of 20 or 100 mg. per kilogram, the pH of the blood was similar to that after 15 minutes but the bicarbonate in the plasma had decreased significantly. Consequently, pCO_2, which increased after 15 minutes, came back to normal. pH and bicarbonate in the aqueous showed no change after 15 minutes. However, owing to the decrease of bicarbonate in plasma, the excess of bicarbonate in the aqueous was almost the same as in untreated controls. The same type of experiments repeated on animals three hours after nephrectomy showed that in the untreated animals the pH and bicarbonate in blood and aqueous humor were significantly below those in normal animals and, again, that the concentration of bicarbonate was significantly above that in the blood and similar in value to that in normal rabbits. Thirty minutes after administration of 50 mg. of acetazolamide per kilogram the pH of blood and aqueous humor dropped below that in untreated animals. The bicarbonate concentrations in both fluids were higher than in untreated animals and, again, the aqueous-to-plasma ratio of bicarbonate was normal. The effect of acetazolamide on pH and total CO_2 was also tested in animals after administration of ammonium chloride, 270 mg. per kilogram. Bicarbonate in the aqueous two and one-half hours after the administration of NH_4Cl exceeded again that in the arterial plasma, but the excess was less than in the untreated animals, and pH and bicarbonate in plasma as well as in aqueous were well below those in untreated animals. If, in addi-

tion to ammonium chloride, acetazolamide was given intravenously, the ratio of aqueous to plasma of bicarbonate was similar to that in untreated animals. Acetazolamide had no significant effect on the aqueous-plasma ratio of sodium, neither 15 minutes nor 2½ hours after administration of acetazolamide. The effect of acetazolamide on the transfer of ascorbic acid across the blood-aqueous barrier was studied by injecting the acid intravenously so that its level in plasma was 5 to 10 mg/100 ml., well in excess of the level of 2 to 3 mg. at which maximum levels in aqueous are obtained. The aqueous humor from one eye was removed three hours after the initial injection, at which point a steady state prevailed, and the concentration of ascorbic acid was found to reach 70 mg/100 ml. When acetazolamide or ammonium chloride was given and the ascorbic acid in the aqueous of the second eye was determined three hours later, no change in the distribution between plasma and aqueous was found. The effect of acetazolamide on the time of appearance of fluorescein was studied in a rabbit anesthetized with urethan; 15 minutes later 1 ml. of a 10% solution of sodium fluorescein was injected intravenously into the marginal ear vein within a period of 10 seconds. It took about four minutes for fluorescein to pass in a significant amount into the anterior chamber. In 10 rabbits the mean time for fluorescein to appear in the area of the anterior chamber immediately adjacent to the lens was 248±26 seconds. The ability of acetazolamide to modify the appearance time was studied 15, 60, 180, and 360 minutes after the injection. At the end of the first two periods the appearance times were significantly above the mean value of the control series, but later this difference disappeared, although acetazolamide was administered at hourly intervals. The same result was obtained whether 5 or 50 mg. of acetazolamide per kilogram was used. Nephrectomy itself had no significant effect on time of appearance, but ammonium chloride increased it to 335±30 seconds. The measurements of the intraocular pressure were carried out with the Schiøtz X tonometer. In anesthetized rabbits the intraocular pressure fell slowly during a period of five hours. In conscious animals, however, it remained constant during this period. After administration of acetazolamide, 100 mg. per kilogram at once or 50 mg. per kilogram followed by 25 mg. per kilogram at hourly intervals, the pressure was brought down in 30 to 60 minutes by two to three divisions, but later there was a significant recovery towards the initial values. Ammonium chloride produced a similar effect; however, the drop in the pressure remained low over a period of five hours. The authors point out that their results contradict the results obtained by Green and his associates and agree, as far as the effect of acetazolamide on the pH and bicarbonate on the aqueous is concerned, with those of Becker. Their observations, however, on the effect on the pH and bicarbonate of the plasma disagree with the findings of Becker and of Becker and Constant, and lead to the conclusion that the ratio of aqueous to plasma of the bicarbonate is not changed by acetazolamide. They point out that the increase in the rate of formation of aqueous demonstrated by the fluorescein method, under the influence of acetazolamide, is only temporary, as the drug inhibits the carbonic anhydrase. They conclude that the activity of this enzyme cannot be essential to the accumulation of excess bicarbonate in the aqueous humor. The constancy of the steady-state ratios for sodium, hydroxyl, and bicarbonate ions, according to the authors, therefore, lends support to the experimental evidence that the intraocular dynamics are not essentially effected by acetazolamide. The disagreement with findings of Becker, who found that the rate of flow of the aqueous humor was depressed 60% by acetazolamide in conditions of steady state is explained by the authors by the fact that Becker used in his experiments nephrectomized rabbits in which the permeability of the blood-aqueous barrier was not normal. The decrease in the rate of flow of the aqueous humor under

the influence of acetazolamide is explained by a disturbance of the normal osmotic balance between the aqueous humor and the blood, which leads to a loss of bicarbonate. This loss is responsible for the temporarily decreased rate of formation. The transitory character of the depression of the ocular pressure is in agreement with the findings of Becker and of Becker and Constant. The prolonged decrease in the intraocular pressure observed in glaucoma patients may be due to the fact that certain homeostatic mechanisms which regulate the intraocular pressure are operating at normal levels of pressure and are eliminated or inadequate at higher pressure. This seems to be confirmed by the fact that a depression of the intraocular pressure in glaucoma by miotic drugs removes also the depressing effect of acetazolamide on these eyes. The mechanisms by which acetazolamide apparently modifies the osmotic balance between the blood and the aqueous humor appears still unsolved but may be related to an increased acidity of the eye, which is only temporary. This could be due to a disproportionate inhibition of carbonic anhydrase in different layers of the eye or to homeostatic mechanisms.

Similar experiments were carried out by Green and his associates,[37] who studied the effect of an intravenous injection of a single dose of acetazolamide upon the pH and bicarbonate concentrations of the blood plasma and of the anterior and posterior aqueous of the rabbit eye over varying periods of time. Their results, however, do not agree completely with those of Langham and Lee. The discrepancies so far could not be explained. Acetazolamide, 30 mg. per kilogram of body weight, was given to adult rabbits. Samples of 20 to 30μl. of posterior aqueous and 0.1 to 0.2 ml. of the anterior aqueous and blood plasma were taken. The blood was heparinized. The fluids were drawn directly from the animal to the glass cell of the pH meter so that the pH could be measured under anaerobic conditions. The pH values were corrected to 37 C, with use of the correction factors of Langham and Lee. The initial value for the bicarbonate and pH (for which different animals were used) was determined in the aqueous of the one eye, while the aqueous of the other eye was analyzed at the indicated time period. In control experiments it was shown that paracentesis of one eye did not effect the values in the other eye. In the posterior chamber the bicarbonate concentration fell very fast, with a maximum decrease of 21% in 60 minutes, and returned to normal after 3 hours. In the anterior chamber the bicarbonate decreased first very slowly and reached a minimum of 17% to 19% below the original initial value after about two hours. After a transient slight increase in the third hour, it went back to 80% of the original and remained so until the end of the eighth hour. It took about eight hours more for recovery. In the plasma the bicarbonate was unchanged in the first three hours after acetazolamide injection and then also decreased by 22% in the following three hours and returned to normal after eight more hours. The pH in the anterior chamber fell slightly during the first two hours and continued to fall for the next four hours, while in plasma the situation again was different, as the pH remained unchanged for the first five hours after acetazolamide administration and a slight drop occurred only during the sixth hour. The authors discuss exhaustively the possible significance of these results on the dynamic of the aqueous formation. They point out that the lag between the plasma and the aqueous, as far as the decrease of the bicarbonate under the influence of acetazolamide is concerned, cannot be interterpreted unequivocally and that the action of the drug upon the eye is not secondary to renal or systemic effects. The return of the bicarbonate level to normal in the aqueous at a time when it is going down in the plasma, however, speaks strongly in favor of a direct effect of acetazolamide upon the elaboration of bicarbonate into the posterior chamber. Its transient rise in the third hour after acetazolamide injection in the

anterior chamber can be explained as the result of the changes of concentration in the posterior chamber, but the subsequent fall suggests that some factor other than the level in the posterior chamber is the major determinant of the level in the anterior chamber, and as this secondary drop coincides with the decrease in bicarbonate in blood, it suggests that, in addition to the secretion into the posterior chamber of bicarbonate, the diffusion between the anterior chamber and the blood is a major factor in the elaboration of bicarbonate concentration in the aqueous. The results of the authors differ from those of Langham and Lee as far as the effect of acetazolamide on the pH of the aqueous is concerned, and the authors find no support in their results for the assumption that the drop in the bicarbonate in the posterior chamber during the first 60 minutes after acetazolamide administration is the result of changes in the pH. On the other hand, they point out that, while the initial drop in the bicarbonate concentration in the posterior chamber could be explained by the concomitant inhibition of the carbonic anhydride by the drug, the continuation of this low level three to six hours after the injection, when 50% of the carbonic anhydride activity has been restored, is incompatible with the concept that this enzyme is responsible for the rate of secretion of the aqueous.

Becker[38] investigated the role of ascorbic acid in maintaining the bicarbonate excess in the aqueous humor of the guinea pig, as well as the effect of acetazolamide on this concentration. Of three groups of guinea pigs, one received a scorbutic diet; a second, a scorbutic diet plus 50 mg. of sodium ascorbate daily subcutaneously (scorbutic controls), and the third was kept on a normal diet. In scorbutic animals, the level of ascorbic acid in the aqueous dropped to 1/10 of its original value after 12 to 22 days. The controls with ascorbic acid supplement gained 100% to 200% in the aqueous. Bicarbonate was determined in the posterior and anterior chambers as well as in heparinized cardiac blood by determining the total carbon dioxide and calculating the bicarbonate from pH values found for normal guinea pigs. Acetazolamide, 70 mg. of acetazolamide per kilogram was initially injected intraperitoneally, followed by 35 mg. per kilogram six times an hour. The bicarbonate in the posterior chamber was found to exceed the plasma value by 54%, while in the anterior chamber the excess was 43%. The scorbutic diet had no effect on the bicarbonate concentration, nor did the ascorbic acid excess influence these values. This suggests that ascorbate is not necessary for maintaining the bicarbonate excess in the aqueous and is not an important factor in determining the rate of aqueous flow. Acetazolamide injections decrease bicarbonate in both chambers. In the posterior chamber the excess over plasma was lowered to 30%, and in the aqueous, to 19%. These changes are consistent with an average decrease in the K_f:K_a ratio of 58%. The author points out that the failure of the ascorbate to alter the bicarbonate in the aqueous is consistent with the lack of change in the anterior chamber concentrations of sodium and chloride in scorbutic animals. The effects of acetazolamide are similar to those found in rabbits, and the perfusion data in guinea pigs which indicate that the fall in the intraocular pressure occurs without alteration in outflow facility in this species, as in the rabbit and human, suggest, according to the author, the need for carbonic anhydrase for the maintenance of the bicarbonate excess in the aqueous of the guinea pig.

Green and associates[39] investigated the steady-state distribution and concentrations of the bicarbonate in the anterior and posterior chambers when its level in the plasma was increased from about 20 mM per liter to about 30 mM per liter. This increase in the level was obtained by intravenous injections of 6 to 8 ml. per 10 minutes of 1.3% $NaHCO_3$ into rabbits. To determine the effect of this higher level of bicarbonate on the exchange of water in the anterior chamber, the intraocular pressure of the anesthetized rabbit eye was measured with a Schiøtz tonometer and tonography

was performed with a Müller electronic tonometer. The maximum level of bicarbonate in blood plasma was reached after 60 minutes and maintained at a steady level for 4 hours. The maximum in the anterior and posterior chambers was somewhat delayed but remained also constant later for four hours. The concentration in the anterior chamber at this stage was 35 mM per liter, and in the posterior chamber, 44.1 mM. The method used for the determination was the usual procedure with an accuracy of ±5%. There were no changes in the intraocular blood pressure, and the results of tonography indicated also that there was no change in the rate at which the aqueous fluid left the anterior chamber. The authors point out that in the steady state at the increased level of bicarbonate in plasma the ratio between the concentrations in the posterior and anterior chambers was the same as under normal conditions. However, the ratio of concentrations in both chambers to that of plasma was significantly lower. Under these conditions, the ratio of the rate of flow as a fraction of the volume of anterior chamber to the rate of diffusional exchange per minute between the anterior chamber and blood plasma is not constant with increasing bicarbonate level in the plasma. The authors conclude, therefore, that the source of the bicarbonate ion in the aqueous humor of the anterior chamber is not adequately defined by the current concepts based upon diffusional exchange with the blood and the differences between the quantities entering by flow from the posterior chamber and leaving at the angle.

Maurice [40] determined the exchange of Na^{24} between the vitreous and the aqueous body and the blood stream. This investigation appears of particular interest in view of the fact that the exchange between the aqueous and the vitreous seems to play a major role in the dynamic of the aqueous formation. Sterile solutions of $Na^{24}Cl$ containing a little fluorescein were injected in amounts of 0.01 to 0.02 ml. into the vitreous in such a way that the injected fluid was located halfway between the retina and the posterior lens surface. The rabbits, of mixed stock, under general anesthesia (ethyl chloride) were in one series of experiments killed at a determined period, and aqueous and vitreous samples were removed and their radioactivity was assayed in an F-10 liquid counter. To correct for the reentry of the labeled Na from the blood, the same determinations were carried out on the noninjected contralateral eye. In a second series of experiments, the amount of Na^{24} which remained in the eye at various times after injection was determined with the help of an external Geiger-Müller counter. The radioactivity of the active material and variations in counter sensitivity were tested by measurements on a drop of the injected material dried on filter paper. Finally, the conductivity of the vitreous and the aqueous was measured in a series of experiments with platinum wire electrodes by means of a 2.9 kc/s bridge. The injection was found to produce no significant changes in the eye except for an increase of the intraocular pressure to 32 mm. after the injection of 0.01 ml. and to 79 mm. after injection of 0.02 ml. After a delay of one hour, the Na^{24} increased in the aqueous to reach a maximum after six hours, and this level was preserved for a considerable time. The ratio of the concentrations in the aqueous and in the vitreous in this steady state was 0.020 ± 0.01. After the steady state was reached, Na^{24} disappeared from the vitreous at an exponential rate. The turnover rate at that point was calculated to 1.56×10^{-3} $min.^{-1}$, in excellent agreement with values found by Friedenwald and Becker for the rate of penetration of Na^{24} into the vitreous humor from the blood. The conductivity of the vitreous was in all measurements 3% to 20% below that in the aqueous, with a medium value of about 10%. The mathematical treatment of the dynamic of the vitreous-blood exchange, made under the assumption that the shape of the vitreous body is roughly that of a sphere with the center removed, gives a transfer coefficient which is 10 times greater than the experimentally determined one. As the conduc-

tivity of the vitreous does not differ significantly from that of the aqueous, the author concludes that the limiting factor for this transfer is the resistance to diffusion of a membrane in the coats of the globe of the eye, and on the basis of the estimation of electrical resistance of various parts of the eyes the authors arrive at the conclusion that the resistance to the vitreous-blood exchange is located in the external limiting membrane of the retina. He also draws attention to the fact that in the rabbit only 5% of the total posterior surface of the vitreous body contains retinal vessels and that in animals with a more extended vascularization of the retina the coefficients controlling the vitreous blood exchange may be very different. The calculation of the rate of the appearance of Na^{24} in the aqueous, carried out under the assumption that the part of the vitreous which the labeled material has to pass is a flat cylinder with a radius equal to the distance between the site of injection and its border with the posterior chamber, yields theoretical values in good agreement with those observed. The author gives a detailed mathematical treatment of the dynamic of the exchange between the vitreous and the aqueous and compares his results with the experimental data of his own with those of Kinsey and Parron on labeled Na^{24} and thiocyanate. This mathematical analysis of the initial stage of the vitreous-aqueous exchange offers a possibility to eliminate certain difficulties in the mathematical treatment of the dynamic of the aqueous formation of Friedenwald and Becker.

Davson and Luck [41] determined the influence of acetazolamide on the turnover of Na^{24} injected systemically, in the aqueous and cerebrospinal fluid, and on the steady-state distributions of sugar, chloride, and bicarbonate between plasma and these fluids. Rabbits, dogs, rats, cats, and monkeys were investigated. Na^{24} was given either by continuous intravenous injection or by a single intraperitoneal injection. Acetazolamide, 100 mg. per kilogram of body weight, was given intravenously either one hour or immediately before the infusion began. The aqueous was removed immediately before acetazolamide injection from one eye and two and one-half hours later from the other. Acetazolamide was found to be without significant influence on the rate of turnover of Na^{24} in the aqueous but to depress the turnover in the cerebrospinal fluid by about 40%. The maximum decrease consistent with the experimental data for the aqueous could have been 6.5%. This seems difficult to reconcile with the generally held opinion that acetazolamide decreases the rate of secretion of the aqueous by more than 60%. The authors point out, however, that, in presence of such a slowing down of the aqueous flow, the diffusion component of the aqueous-blood-barrier exchange is very high and difficult to assess and, therefore, that relatively small changes in the turnover rate of Na^{24} can result in spite of a visible inhibition of the secretion of the aqueous. The different behavior in the cerebrospinal fluid after acetazolamide injection is held to be due to a smaller role of the diffusion factor, as the diffusional exchange of ions between plasma, on the one hand, and the cerebrospinal fluid and extracellular fluid of the nervous tissue, on the other, takes place at about the same rate, so that no appreciable gradients of concentration of Na^{24} between these two fluids are present during the approach to equilibrium. Acetazolamide was found to be without influence on the steady-state distribution of sugar in either the aqueous or the cerebrospinal fluid. In the case of ions the steady state distributions were very differently affected by acetazolamide in individual species. In those species in which the concentration of the ion in the aqueous exceeds that in the plasma, injections of acetazolamide tended to depress the concentration of the ion. The concentration of ions which was normally lower in the aqueous, on the contrary, in general was increased. The effect of acetazolamide, therefore, was to shift the levels of the ions in the aqueous in the direction of the levels in the plasma. The authors suggest, therefore, that acetazolamide mainly

may affect quantitatively and possibly qualitatively the composition of the secreted fluids rather than to depress the flow itself, although both factors may be influenced. They also point out that the interpretations of the decrease in the intraocular pressure caused by acetazolamide if due to the decrease of secretion of the aqueous is confronted with several difficulties and cannot be regarded as completely conclusive.

Harris and associates [42] investigated more extensively the aqueous-to-serum steady-state ratios of potassium and sodium in normal rabbits and rabbits treated with various hypotensive drugs. This investigation was stimulated by incidental observations that these aqueous-to-serum ratios in the individual rabbits were not identical, which suggested involvement of secretion processes in the elaboration of concentrations of these ions. Aqueous of rabbits, monkeys, and humans was examined. The two cations were determined by flame photometry. The animals were only locally anesthetized. The aqueous-serum ratio for potassium was in excess of 1 in the rabbit. The sodium ratio was uniformly lower, with much greater scattering for potassium. In the other species, however, the potassium ratio was below 1. In the monkey the ratios were about equal. In the cat, the potassium ratio was in the 80's, against 0.97 for sodium. The same relation was found in one normal eye for humans, where the potassium ratio was below 0.9. The ratio of 1.03 for potassium in rabbit is at variance with that of 0.89 found by Langham and Taylor, and the reason for this discrepancy could not be found. As the movements of radioactive cations from plasma to aqueous did not indicate any difference in the rate between the potassium and sodium, the author concludes that the distribution of potassium is actively controlled. Results on the influence of ocular hypotensive drugs seem to agree with this conclusion. Acetazolamide led to a decrease in serum and aqueous potassium, with essentially unaltered steady-state ratio, while sodium concentration remained unchanged. The change in potassium required at least .30 mg. of dibenamine per kilogram, while changes of bicarbonate were obtained with as little as 5 mg. of the same drug, which inhibits aqueous formation and which showed effects similar to those of acetazolamide. Two other hypotensive drugs, dichlorophenamide, 30 times as potent an inhibitor of carbonic anhydrase as acetazolamide, and chlorothiazide, a weak carbonic anhydrase inhibitor, were tested. The first drug was a strong hypotensive agent; the second, without effect on the ocular pressure. Correspondingly, the first drug in low dosage produced a fairly marked decrease in serum and aqueous; 5 mg. had the same effect as 50 mg. of acetazolamide. Chlorothiazide had little effect. The author concludes that the formation of aqueous cannot be accounted for solely by the active transfer of one anion or one cation but that possibly the secretion of the whole aqueous is involved and dependent upon the composition of the blood plasma. He points out that the relation between experimentally found changes of bicarbonate and potassium in the aqueous runs in an opposite sense to those found in many clinical and laboratory situations, and this difference may be a reflection of the correlation between hydrogen ion and potassium levels.

Wistrand [43] investigated the effect of acetazolamide administered by intracarotid injection on the intraocular pressure to determine whether the depressing effect of the drug is due to a direct action on the eye or is an indirect one brought about by systemic changes. The intraocular pressure was measured by a manometric method with cannulation of the anterior chamber. As the intraocular pressure of rabbits was found to be very sensitive to cannulation, cats of 2.5 to 5 kg. body weight were chosen for the experiments. Owing to the instability of the intraocular pressure caused by cannulation, the experimentally obtained values showed wide scattering. The author gave a detailed description of the cannulation, the operative procedure, the manometer, and the technique of the intracarotid injection.

Before the start of the experiment the animals were given injections of promethazine to reduce the instability of the intraocular pressure and of heparin to eliminate blood coagulation. After insertion of the eye pressure cannulae, sulfadimidine, an analogue of acetazolamide with no significant effect on carbonic anhydrase, was injected first into both carotids; the infusion syringe only of one carotid was then switched to the container with acetazolamide, so that one eye was perfused with acetazolamide and the control eye with sulfadimidine during the whole experimental procedure. The effect of acetazolamide was measured on the outflow pressure, which was defined as the determined intraocular pressure minus the episcleral venal pressure, which was not measured but assumed to be the same in cats as in man and rabbits, about 13 cm. H_2O. The intraocular pressure on both sides was considered to be stable if for 10 minutes the outflow pressure did not vary by more than 20%. The duration of the acetazolamide perfusion was 15 to 30 minutes. Owing to the wide scattering of the values of the intraocular pressure, the changes in the acetazolamide-perfused eye against the control were not always in the same direction. The mean value, however, from all eight animals was lower than those from controls, and the maximum depression of the mean was reached eight minutes after the start of the perfusion. The mean intraocular pressure decreased during the period of perfusion in both eyes. The decrease in the mean outflow pressure in the acetazolamide-perfused eye was 63%, which agrees with the values obtained by Gloster and Perkins (1955) after intravenous injection of 50 mg. of acetazolamide per kilogram into cats. This corresponded, however, to a drop in the intraocular pressure from the mean of 30.2 to 28 cm. of H_2O, with a range of intraocular pressures between 16.5 and 35.8 cm. of H_2O. The author points out that the negative results of Gloster and Perkins with the intracarotid injection of acetazolamide in the rabbit are probably due to 50 to 300 times as large a dosage per unit time, which increased the acetazolamide concentration in the control eye very rapidly to a level at which it already maximally depressed the intraocular pressure. The author reaches no answer to the question why the subconjunctival administration of acetazolamide failed to show any effect on the intraocular pressure.

Barany and associates [44] investigated the slimy and highly viscous mucinous layer which covers the endothelial surface of the cornea of certain owls. The animal chosen was Strix aluco. Five animals, weighing about 400 gm. each, were used. In a first series of experiments the penetration of fluorescein injected into the anterior chamber was observed under anesthesia with the slit lamp and Zeiss stereodissecting microscope. They observed that the mixing of fluorescein with the aqueous is a much slower process than in ordinary eyes and that there is a gradient of viscosity within the anterior chamber, the more viscous layer being closer to the cornea. It seemed possible that the circulation of the aqueous takes place only close to the lens and iris. This was confirmed by an experiment in which a small amount of India ink was deposited close to the center of the pupil with a needle through the limbus. The ink spread instantaneously over the iris, extending to the chamber angle. The same phenomenon was observed in another set of experiments in which homogenized cream was injected into the posterior chamber with a needle through the posterior part of the eye. The cream appeared in the pupil but did not rise appreciably above the pupillary part, spreading mostly laterally. Later it spread into the bulk of the aqueous, but a clean space was visible close to the cornea. Finally, aqueous humor was removed from the owl's eye in form of an elastic jelly which could be pulled in form of threads. This jelly rapidly liquified in contact with bacterial hyaluronidase. Its relative viscosity measured after fivefold dilution and addition of diethyldithiocarbamate was found to be 1.60 at 20 C and dropped to 1.08 in the course of one hour after addition of 6 viscosity-

reducing units (VRU) per milliliter of a testicular hyaluronidase. The authors suggest that the mucinous layer is produced by the corneal endothelium and is analogous to the mucinous hyaluronidase-sensitive coating on the trabeculae, which probably is also produced by the endothelial cells covering the trabeculae. Its physiological function may be to restrict the circulation of the aqueous to a smaller volume of the anterior chamber, which in the owl appears excessively wide.

Berggren and Vrabec [45] investigated the effect of various hyaluronidase preparations on the morphology in vivo of the trabeculae of the angle of the anterior chamber. The experiments were carried out on cattle, sheep, and rabbit eyes. The anterior chamber was perfused, according to previously described techniques, until the maximum of the resistance flow was obtained, and four different hyaluronidase preparations, namely, a testicular, a staphylococcal, a bee venom, and a snake venom hyaluronidase were injected. As soon as the resistance dropped, the anterior parts of the eyes were immediately cut meridoneally and the eyes were fixed in 10% formalin for 24 hours. Replicas of the trabecular meshwork were then made according to the technique of Vrabec with 5% chloroform solution of methyl methacrylate, and the changes in the appearance of the trabecular and intertrabecular spaces were recorded by inspection by two independently working investigators. Changes were seen in 8 of 10 pairs of cattle eyes and 9 of 10 pairs of rabbit eyes, which made the observation highly significant. They consisted in sharper outlines of the trabeculae and in the increased depth of the lapses between them. The impression was that in the enzyme-treated eyes some substance had been removed. The other preparations of hyaluronidase produced similar changes. To prove that the morphological changes coincide with the drop in resistance, one eye of an animal was perfused one hour longer after the maximum drop in resistance was achieved. There was no difference in morphology between the two eyes. The drop in resistance due to the massage effect was not associated with corresponding morphological changes. On the other hand, when replicas of the trabeculae were made four hours after the perfusion with hyaluronidase was interrupted, the restitution of the coating of the trabeculae, which previously was observed by physiological means, appeared also in the restitution to normal of the amorphous substance in the intertrabecular spaces. This substance proved to be resistant to hyaluronidase. When the replicas were made 24 hours after the restitution instead of 4 hours afterward, the trabecular substance showed characteristic beady formations, the significance of which is unknown. The authors conclude that their morphological studies are in agreement with the physiological experiments of Barany, and their interpretation. They consider that the intertrabecular substance, responsible for the flow resistance of the angle, is composed of several elements, only one of which responds to hyaluronidase treatment. Different preparations of the enzyme with identical activities produce about the same effects, and the hyaluronidase-sensitive substance can, therefore, be regarded as hyaluronic acid. The presence of a hyaluronidase-insensitive material in restitution experiments is due either to an increase of those constituents of the intertrabecular substances which were insensitive to hyaluronidase from the beginning or to the formation of a newly formed constituent. The authors also report that an increase in this hyaluronidase-insensitive substance was observed in the eye contralateral to that in which the treatment with hyaluronidase was carried out.

Böck and associates [46] investigated the presence of a substance in the aqueous of normal persons and patients with simple and inflammatory glaucoma which causes the dilatation of capillaries in the ear of rabbit after cutting of the nerves. This substance was found to be present in nerves of the posterior sensory routes and not be identical with acetylcholine, adenosine phosphate, or

histamine, from which it can be distinguished by the character of its pharmacological effects. The aqueous was injected 8 to 28 days after the nerves of the ears were cut; the effect was estimated by comparison with the other, noninjected, ear. Fresh aqueous did not produce any capillary reactions. This was found to be due to the presence of an enzyme which rapidly destroyed the substance. Aqueous of patients with glaucoma when heated to boiling point before injection showed a definite dilatory effect on the capillaries. This was not the case with the aqueous of normal persons. The aqueous from simple glaucoma cases was much more effective than that from cases of inflammatory glaucoma. The authors believe that this might be due to a higher concentration of the destroying enzyme in the aqueous of the latter cases. Control experiments on the content of choline esterase in the aqueous indicate that the two enzymes are not parallel in the aqueous. The capillary-dilating substance from extracts in posterior sensory routes is also inactivated by the aqueous. This suggests an identity of the capillary-dilating agent of the aqueous with that in the sensory routes. Aqueous of cattle was also found to be free of the dilating substance. The authors suggest that the presence of the capillary-dilating substance in simple glaucoma might indicate a certain role of capillary dilatation which so far had not been discovered in cases of simple glaucoma.

Duke-Elder and Maurice [47] proposed a unified system of symbols to be used in the mathematical treatment of intraocular dynamics.

Berggren [48] checked the findings of Leach and Lloyd (1955 and 1956) that unsaturated aldehyde of the oil of lemon peels, citral, produces in monkeys and rabbits an ocular hypotension and an epithelial damage in the canal of Schlemm or the angular trabeculae. Citral, 10 mg. per kilogram, was injected in 25% alcoholic solution subcutaneously in four rabbits and the intraocular pressure was determined with a Schiøtz tonometer three and six hours later. The first injection was followed after 5 days with repeated injections of higher doses, which were increased after 12 days to 1000 mg. per kilogram. The intraocular pressure was measured daily during 21 days. No changes in comparison with the normal animals were found under the influence of citral.

Siegert [49] reports results of a few experiments on the concentration of total lipids and cholesterol in glaucoma patients as compared with normal controls and cataract patients. The purpose was to obtain indications of whether hypothyroidism is involved in the pathogenesis of glaucoma. No significant difference was found in the cholesterol levels, but the total lipids, with an average value from 77 persons of 1000 mg. %, with variations between 925 and 1037 mg. %, appeared higher than those reported in the literature for normal persons. The difference, however, was much smaller than that found in cases of hypothyroidism.

Lens

Resnik [50] prepared an ultracentrifugally homogeneous α-crystallin from beef lenses and determined its molecular size from sedimentation and diffusion constants. The method of preparation was a combination of the older procedure of Burky and Wood and the most recent procedure of François and associates. The decapsulated lenses were stripped of the cortex in the cold and homogenized in an equal volume of ice-cold distilled water. The homogenate was centrifuged for 30 minutes between 15,000 and 20,000 g, and the supernatant solution was adjusted to pH 5 with HCl. The precipitate was removed by centrifugation, and ethanol was added to a concentration of 13%, at a temperature between 3 and 10 C. This second precipitate corresponded to François' α_2-crystallin. Both precipitates were dissolved in a diluted NaOH solution to a pH between 6.5 and 7 and were reprecipitated two or three times. The solution was dialyzed against distilled water for 24 to 48 hours, during which time

a precipitate appeared which was discarded. The supernatant contained only one component in the ultracentrifugate. Since the material precipitated by 13% ethanol was precipitated at pH 5, the author concludes that the differentiation between α_1- and α_2-crystallin of François is not justified, as the ethanol is only necessary for complete precipitation of α-crystallin at pH 5. S^{20} of the preparations in barbital acetate buffer of pH 7.5 and 0.20 M NaCl was 18.8, and its dependence on salt concentration was not completely consistent with various preparations. There was no difference in S^{20} between α_1, and α_2. Diffusion measurements were carried out at 40 C in a Spinco Model II electrophoresis diffusion apparatus, and the sedimentation constants led, under the assumption of 0.74 for V^{20}, to a molecular weight of 1,000,000, slightly lower than the value reported by François, which was obtained with a light-scattering method. The ratio of the friction coefficients for α-crystallin to the friction coefficient of an anhydrous sphere of the same size was 1.78. The α-crystallin appears, therefore, as an asymmetric molecule.

François and Rabaey[51] continued their interesting studies on the lens protein, which was found in animal lenses to be formed only during the embryonal development and in the first few months of the postnatal life. They have investigated the presence of this protein in human lenses and at the same time have determined the changes in the electrophoretic pattern of proteins from human lenses related to the age of the persons. The electrophoresis was carried out according to the procedure of Grassmann and Hannig with the apparatus Elphor H in a barbital acetate buffer pH 8.6, ionic strength 0.1. They first found that the pherograms from lenses of children showed four components, the fastest sharply separated from the three slower components, which do not separate as clearly from each other. This behavior differentiates lenses of children from lenses of all so-far-investigated mammals, except young gorillas. The fastest fraction resembles, on the basis of its mobility and other properties, α-crystallin from other animals. The results obtained by paper electrophoresis of lenticular extracts from adults were not clear-cut and were very difficult to interpret, as in lenses from middle-aged persons, for instance, no clear separation whatsoever of any fractions could be obtained, but all the proteins seemed to migrate in bulk. This suggests that the adult lens contains proteins which differ from those in the young lens and that with the age the proteins of the young are subjected to chemical transformations. The authors then studied the transition from the pattern seen in young children of about 3 months of age to that of young adults in their 20's. They found that as early as at the age of 10 years the characteristic pattern of the younger children is almost completely lost, and they stress these rapid senile changes, which do not take place at all, for instance, in lenses of horses, even at the age of 15 years. The changes observed in the first few years of life mainly concern the slowest Fraction 4, which decreases continuously, while Fraction 2 increases at the same time. This development seems to continue later, although quantitative measurements are extremely difficult after 10 years of age. A series of observations was also made on the protein composition in various layers of the human lens. These determinations also have bearing on the problem of senile changes, as the nucleus contains older fibers than the cortex. Furthermore, the determinations on individual layers of the lens provide clearer patterns which are easier to evaluate and determine quantitatively. As could be expected, there was little difference in the patterns between the cortex and nucleus of lenses from normal infants and from prematurely born infants. The small differences which were observed concerned α-crystallin, which was somewhat decreased in the nucleus, and mainly Fraction 4, which was considerably higher. This trend also showed up when lenses were divided into three or four layers. In this type of

fractionation, differences could also be observed between Fraction 2 and Fraction 3, the latter somewhat predominating in the nuclear part. If the percentage of the first three fractions is calculated, with elimination of Fraction 4, it is seen that the quantity of Fraction 1 remains constant throughout the various layers of the young lens, while Fraction 2 predominates in the cortex as compared with Fraction 3, which predominates in the nucleus. A quantitative fractionation was to some extent still feasible in adults aged 24 years. It was found that Fraction 4 is significantly lower at this age than in neonatal lenses. This loss in Fraction 4 is compensated by an increase in Fractions 2 and 3, while Fraction 1 shows about the same percentage. The comparison of the total amounts of protein in lenses of 24-year-old persons and neonatal lenses shows that Fraction 4 is not synthesized at all from the end of infancy on, while the other protein fractions increase percentually to about the same extent. This, therefore, demonstrates the presence also in humans of a protein which is formed only during the embryonal period and the early stages of infancy. In agreement with that, it was found that Fraction 4 in young adults is localized in the nuclear part of the lens. This increase in Fraction 4 is at the expense of α-crystallin. The ratios between Fractions 2 and 3 follow the trend found in neonatal lenses. To test how far the four protein fractions found in human lenses can be regarded as homogeneous fractions, a series of experiments were carried out with a newly developed method of ultramicro electrophoresis in agar gel, which makes it possible to investigate minute quantities of proteins and to obtain electrophoretic patterns of embryonic lenses during the initial stages of evolution; 0.1μg. of protein can be analyzed with this method, which is described in detail. The quantitative evaluation of the patterns is carried out with the aid of a sensitive densimeter, and the density curves are integrated with the aid of planimetry or by weighing the prospective zones. The method permits one to study simultaneously electrophoretic patterns of several samples which are to be compared, and the conditions can be arranged in such a way that, for instance, only α-crystallin migrates to the anode, while all the other fractions migrate toward the cathode, which makes the separation particularly clear. With this method it was possible to split the lenticular protein of an infant into at least 10 different components, which are distinguished as 2 rapid, 3 slow, and 5 middle fractions. The rapid fractions were found to increase with decreasing age of the lenticular fibers. Among the five middle fractions, Fraction 4 is predominant in infants and shows a relative decrease before the age of 4 years. Among the slow fractions, the slowest also decreases in quantity during the first few years of life and is also slower in the cortical part as compared with the nuclear part. In the adult lens the patterns in agar electrophoresis are entirely dominated by the middle fractions. With senescence, the middle Fractions 1 to 3 come to the fore, as compared with the Fraction 4 which was predominant in the infant. The authors point out that the interpretation of changes with the age is made difficult by the conversion of soluble proteins to insoluble albuminoid with progressing age.

Orzalesi and Miglior [52] report about further experiments with the precipitation of lens proteins of the rabbit with basic proteins like protamine, lysozyme, and histones. They studied the effect of these proteins on electrophoretic patterns of rabbit lens proteins. The electrophoresis was carried out according to the technique of Grassman and Hannig at pH 8.9 in barbital buffer, ionic strength 0.1. The pH was around 7.3. They showed that addition of basic proteins at sufficient concentration eliminates completely α-crystallin from the pherograms. At lower concentration one part of the α-crystallin is still visible. If an excess of basic protein is added, these proteins migrate to the cathode. At the right concentration a complex between all the α-crystallin and the basic protein is

formed which precipitates. When serum is added to the lenticular extract containing protamines, the latter are digested by the protaminase of the serum. This leads to a dissolution of the precipitate, and when the supernatant is then subjected to electrophoresis α-crystallin, which previously disappeared from the supernatant of the precipitation, reappears. The authors point out the similarity between the behavior in this respect of α-crystallin and the α-globulins of serum and report that when mixtures of lenticular homogenates and serum are subjected to paper electrophoresis the α-globulin and α-crystallin appear as one spot. They stress the possible significance of these findings for the etiology of certain forms of cataracts, as basic proteins had been previously shown by the authors to be able to produce precipitation in the lens in vivo after having been injected into the vitreous.

The increase of cystine in lens proteins with progressing age and in cataracts suggested to Merola and Kinoshita [53] the idea of investigating the ability of SH groups of lens proteins to undergo oxidation. The experiments were carried out on homogenates of lenses of freshly slaughtered cattle which were prepared in a Waring Blendor at 5 C under nitrogen in isotonic tris buffer at pH 7.4. One lens was homogenized in 20 cc. of buffer, and the homogenate was separated from the albuminoid by centrifugation at 10,000 g for 15 minutes at 5 C. As oxygen itself has no effect on the SH groups of lens proteins and oxidation by hydrogen transfer to some easily reducible substances present in excess involves the danger of secondary protein changes, the reactivity of SH groups was measured with use of catalytic amounts of Cu^{++} (2×10^{-5} M) in pure oxygen. Experiments were carried out at 25 and 37 C. Sample aliquots of the homogenates were taken out at one-hour intervals, and SH groups were determined by amperimetric titration. The thiolic groups of glutathione were determined in the filtrates from precipitation of proteins with sulfosalicylic acid by the nitroprusside method. The oxidized glutathione was determined after electrolytic reduction to reduced glutathione. The method of Roe (1954) was used for the determination of ascorbic acid. Glutathione was found under these conditions to be completely oxidized in one hour, while it took five hours to oxidize it completely in absence of cupric ions. It could be recovered from its disulfide form by electrolytic reduction. In contrast to the ease with which glutathione is oxidized, the thiolic groups of proteins were found under the conditions of the authors to be very little accessible to oxidation. At 25 C as well as at 37 C no more than 7% of the total amount was found to be oxidized in five hours. That glutathione in these experiments did not protect the proteins was shown by removing the first one on a Dowex 1 column, which did not remove any proteins. The same results were true with experiments on dialyzed homogenates. These results suggested that the thiolic groups of proteins are masked in some way and not available for oxidation. This was borne out by experiments in which a reversible denaturation of these proteins was achieved with 8 M urea solutions. Under these conditions the oxidation of thiolic groups of protein was markedly increased, much more at 37 C than at 25 C. Preliminary experiments have shown an increase also in the oxygen uptake of the homogenate. At the same time, however, the interesting and so-far-unexplained observation was made that in presence of 8 M urea the oxidation of glutathione was markedly inhibited, although urea in absence of proteins did not affect the oxidation of glutathione by cupric ions. The authors conclude from these experiments that during the aging and formation of cataract denaturation processes occur in the lens which unfold the protein chains and make their thiolic groups more available for oxidation.

Roberts [54] investigated whether rabbit antisera against rat kidney glomeruli, which, according to Cruickshank and Hill (1953),

combine with other basal membranes in the rat, show also a similar affinity for structures in the eye. The method for the demonstration of the antigen-antibody combinations was that of Coons and Kaplan (1950), based on labeling of the antibodies with fluorescein isocyanate and demonstration of the labeled product by green fluorescence in ultraviolet light. Whole glomeruli, prepared according to Hill and Cruickshank (1953), were injected intraperitoneally into rabbits, six doses at weekly intervals. A globulin fraction from the antiserum as well as from a normal control serum of rabbits was prepared by precipitation with 50% ethanol at −10 C. Both globulin fractions were then coupled with fluorescein. Frozen sections of the rat eye and rat kidney were prepared and incubated with the fluorescent, normal, and antirat glomerular globulins for two and one-half hours at 37 C. After removal of the surplus globulin, the sections were dried and their fluorescence in 360 mμ light was examined. The most pronounced fluorescence was found in the lens capsule, which by itself did not show any disturbing autofluorescence. Fluorescent membrane between epithelium and stroma was also seen in the iris and beneath the ciliary epithelium and within the ciliary body. The structures in the posterior segment and in the cornea could not be examined because of nonspecific staining and autofluorescence. The binding of the antirat glomerular globulin by the lens capsule indicates that the lens capsule is a structure analagous to basal membranes, as was suggested in 1954 by Dische, similarity which also appears in the composition of the characteristic polysaccharides found in the lens capsule and in the reticulum structures.

Mizukawa and Takagi [55] studied the antigenicities of various proteins of the lens. They found that the albuminoid is a much stronger antigen than soluble proteins and is of the Arthus type, while the soluble proteins are of the tuberculin type. The polysaccharide of the lens capsule was antigenic in presence of lens proteins.

Suzue [56] prepared extracts of lens capsule of beef polysaccharides, which proved to be antigenic in rabbits by precipitin and skin-test reactions. A mixture of lens proteins enhanced the antigenicity of the polysaccharides.

This author [57] also confirmed by paper chromatography the previous findings of others that the polysaccharide of the lens capsule contains galactose and glucose and no hexosamines.

Zeller and Devi [58] determined the concentration of exo- and endopeptidases in human normal and cataractous lenses as well as in lenses of rabbits with mature x-ray cataracts and corresponding normal controls. The exopeptidases were determined by the rate of liberation of free amino acids from glycyl-L-leucine (GL), L-leucylglycine (LG) and L-leucylglycylglycine. The liberated amino acid was measured by the amount of oxygen which was consumed in the solution in presence of the L-amino acids oxidase of snake venom which oxidizes very rapidly L-amino acids. The endopeptidases of chymotrypsin character were measured by manometric determination of the hydrolysis of L-phenylalanine ethylester and that of trypsin character by that of benzoyl-L-arginine ethylester. The human lens was found to contain considerable N exopeptidase activity which was not reduced in cataract. A marked drop, on the other hand, was seen in the chymotrypsinase of the human lens. By contrast, the endopeptidase of rabbit lenses was much lower in cataract due to x-rays. This was also true for chymotrypsinase activity.

Ambrosio [59] found carbonic anhydrase in the capsule as well as in the cortex and nucleus of the lens.

Waley [60] continued his important investigations on acid peptides of the lens. In a first series of experiments ophthalmic acid was obtained in the crystallin state and another peptide was found, the composition of which was identified as that of the next lower homologue of ophthalmic acid. The peptide is called norophthalmic acid. The isolation of ophthalmic acid was achieved

by the combination of absorption of this compound on an anion-exchange resin, and its subsequent separation from contaminating phosphorus-containing compounds on a cation-exchange resin, with final chromatographic fractionation on paper and elution of the separated ophthalmic acid. The peptides of calf lenses macerated with water were exhaustively absorbed by stirring with three batches of Amberlite IR-4B acetate, and the resin was transferred to a chromatographic tube of 4 cm. diameter and eluted with 2 N HCl, 250 ml. per hour for three hours. The HCl was first removed by shaking of the eluate with N-methyl-dioctylamine in $CHCl_3$, and the excess base was then removed with $CHCl_3$. The cation-exchange resin was Dowex 50 (200-400 mesh) 12% cross linked. The eluate from the anion-exchange column was percolated through this column, and elution was effected with 3% NH_3 solution. Four fractions of the eluate which contained ninhydrin-positive material were combined to about 3 ml. This solution, which contained ophthalmic acid and other acid peptides, was subjected to paper chromatography with phenol as solvent and developed with ninhydrin. The spots with the R_f of that of alanine were eluated and evaporated to a brown syrup, which was dissolved in water. This solution was treated with 0.3 M copper acetate solution, and the crystallin copper salt of ophthalmic acid which separated (6 mg. from 100 gm. of calf lenses) was collected. A warm solution of 8-hydroxyquinoline in $CHCl_3$ was added to the powdered copper salt and suspended in water, and, after removal of the $CHCl_3$ layer, the extraction with $CHCl_3$ was repeated until the layer was colorless. For further purification the $CHCl_3$ layers were extracted with water and the combined aqueous layers, after threefold extractions with $CHCl_3$, were concentrated to a small volume and diluted with ethyl acetate till turbid. The initial precipitate was spun down, and the crystallin ophthalmic acid was separated slowly from the supernatant. Ophthalmic acid was completely hydrolyzed in eight hours by carboxypeptidase, and the products of hydrolysis were identified by paper chromatography in butanol acetic acid. Two spots appeared, corresponding to glycine and to γ-L-glutamyl-DL-α-aminobutyric acid, which was identified by comparison with the synthetic dipeptide. The paper chromatogram of the brown syrup containing ophthalmic acid showed a spot immediately following that of ophthalmic acid, which, after elution and repeated paper chromatography in butanol acetic acid, was freed from ophthalmic acid and treated with 1-fluoro-2-4-dinitrobenzene in aqueous ethanol containing triethylamine carbonate. Glutamic acid was found to be the end-terminal amino acid in this peptide, while glycine was split off as C-terminal amino acid by carboxypeptidase. Alanine was the third amino acid in the tripeptide, which thus appears as the lower homologue of ophthalmic acid. The author points out the similarity of structure of ophthalmic acid and norophthalmic acid with that of glutathione and the fact that these two first tripeptides have not been found so far in tissues other than the lens.

After the elucidation of the composition of ophthalmic acid, there still remained the problem of whether the glutamyl is bound in the γ- or α-form. The author [61] decided this by comparing the mobility of ophthalmic acid in paper electrophoresis at pH 4 with that of various γ- and α-glutamyl di- and tripeptides. It was found that the γ-peptides have about twice as high a mobility in pyridine acetic acid buffer than the α-peptides. Ophthalmic acid in this respect behaved like the γ-peptides. The final proof of the structure was achieved by a synthesis of ophthalmic acid from benzoxycarbonyl-L-α-amino-*n*-butyrylglycine ethyl ester prepared by the mixed-anhydride method. It was separated from the α-isomer by fractionated extraction from an organic solvent by aqueous alkali. This synthetic tripeptide was found to be indistinguishable from the ophthalmic acid from the lens by the use of paper chromatography and paper electrophoresis, as well as by the x-ray powder

diagrams of the free tripeptides. The author points out that the similarity between the structure of ophthalmic acid and norophthalmic acid and glutathione suggests some important biological role associated with this structural similarity.

Klethi and Mandel[62] carried out a complete chromatographic analysis of nucleotides of the lens of the calf eye. The eyes were taken out immediately after death, frozen, and homogenized in 0.6 N $HClO_4$ at 0 C. The acid was removed by neutralization with KOH at −5 C, and the precipitate was centrifuged off. The supernatant was fractionated on Dowex 1 (200-400 mesh), according to Schmitz (1954). Identification of individual peaks of the chromatogram was carried out by the absorption curves in the ultraviolet; P determinations, according to Briggs; pentose, according to Mejbaum, and paper chromatography with isobytyrate ammonia (100:60), propanol ammonia water (60:30:10), and tertiarybutanol CHl 0.3 N. UDP glucose and galactose were identified by paper chromatography with pyridine ethyl acetate H_2O; UDP acetyl glucosamine, by the reaction according to Aminoff; the pyridine nucleotides, according to Colowick. Of the total nucleotides, 54% were found to be adenine nucleotides, of which 71.5% was ATP and 10.4% was MP. In addition, the two pyridine nucleotides and the three uridine and guanine nucleotides were found, one peak corresponded to CMP.

Dardenne and Pütter[63] determined the concentration of CoA in individual lenses of calf, cattle, pig, and human. They found in the calf, as the average of 12 determinations, 25.4μg. (21.1μg. to 28.2μg.) per gram; in cattle, only 16.2μg. (13.2μg. to 21.2μg.) per gram, and in the pig, in 4 determinations, 19.5μg. (17.5μg. to 22.3μg.) per gram. In young normal human lenses (16 to 21 years), in three determinations, the value was 33.3μg. (30μg. to 37μg.) per gram; in old persons (49 to 60 years), 21.1μg. (16μg. to 27μg.), while in cataractous lenses, in five determinations, only 7.7μg. (3.1μg. to 15.2μg.) per gram. The authors stress the high level of coenzyme A in the lens as compared with the muscle in rabbits, in which only half as much CoA was found as in the calf lens. The high values in younger persons as compared with older ones were found to be due to differences between the cortical and nuclear parts of the lens, the latter containing even in the calf only about half as much as the first. This indicates, according to the authors, an involvement of coenzyme A in the metabolism of the lens. As the citric acid cycle and the fat metabolism were found to play a minor role in the lenticular metabolism, they suggest that the CoA in the lens plays a major role in the very active protein metabolism, as indicated by the high turnover rate of the lens components.

Kinoshita and Masurat[64] carried out experiments to illustrate the possible relationship between glutathione and carbohydrate metabolism and to establish the conditions of the stability of the glutathione in the lens and its possible binding by lens proteins. In the first series of experiments the hypothesis was tested that the activity of the glutathione reductase is coupled with the dehydrogenases of the shunt pathway in the lens insofar as it supplies the latter with the oxidized TPN necessary for the oxidation of glucose-6-phosphate, and in this sense replaces the function of the cytochrome system in respiratory processes. In these experiments the effect of oxidized glutathione on the oxidation of the carbon 1 of glucose was examined by measuring the labeling of CO_2 produced during the incubation of a lens homogenate with C^{14}-glucose labeled in carbon 1. The incubation of cattle lens homogenates was carried out in phosphate buffer, in Krebs-Ringer solution, or with addition of bovine aqueous humor. It was found with the first buffer that oxidized glutathione increased the oxidation of carbon of glucose by 88%. This was not the case with the two other media, which the authors explain by assuming that the reoxidation of TPNH in phosphate buffer is not as rapid as its formation by the dehydrogenases of the shunt mechanism and,

therefore, that the increase in the oxidation of TPNH by oxidized glutathione increases also the oxidation of glucose. This somewhat surprising explanation appears to be consistent with the observation that oxidized glutathione in phosphate buffer increases the amount of $C^{14}O_2$ so that it is equal to that observed in other buffers and by the observation that oxidized glutathione increased oxidation of C 1 also in aqueous humor when N-ethylmaleimide (NEM) was added, which combines irreversibly with SH groups. The measurements of the activity of glutathione reductase by two different methods showed that the turnover in this enzyme reaction is about 10 times as high as the rate of production of TPNH in the shunt mechanism and, therefore, is completely adequate to provide the necessary TPN for the shunt mechanism. The authors show also that this relation between glutathione reductase and the dehydrogenases of the shunt mechanisms holds also for other tissues, with the sole exception of the adrenal gland, in which there is a special situation due to the role of TPNH in the synthesis of steroids. In a second series of experiments the stability of glutathione in lens homogenates was measured by incubating these homogenates at 38 C with glutathione and various other peptides containing glycine and measuring either the relation of free glycine or the increase in the ninhydrin-reactive components. In the case of glutathione the release of cysteinylglycine was also measured. It was found that, while the homogenates under these conditions split peptides which have an α-glutamyl bond, glutathione, which contains the γ-glutamyl bond, resists hydrolysis almost completely. Finally, the physical state of glutathione in the lens was investigated by measuring the extractability of glutathione from rabbit lenses in iso- , hypo- and hypertonic phosphate buffers. In isotonic buffer there was no glutathione passed from the lens to the surrounding medium. In hypo- or hypertonic media, however, there was initially a rapid extraction during the first two hours which dropped sharply in the following two-hour period and finally stopped altogether at a moment when about 20% of glutathione was in the surrounding medium and 80% remained inside the lens. This did not correspond to a state of equilibrium, because the transfer of the lens at the end of the extraction period to a fresh medium did not cause any further extraction of glutathione. The extracted amount could be increased only by adding 3.0 M of urea or 1.5 M of guanidine hydrochloride, which caused much higher extraction. This was not due to the higher tonicity of the medium, as these two denaturing agents could not be replaced by equivalent amounts of glucose or NaCl. The authors conclude from their experiments that 80% of the glutathione is somehow bound inside the lens fibers and not available for diffusional exchange. In addition, there is under normal conditions an impermeability of the lens surface membranes for glutathione which disappears only in hypo- or hypertonic solutions. The possibility should, however, be considered that this unequal distribution of glutathione between the lens and the surrounding medium could be the result of the morphological inhomogeneity of the lens. It must be kept in mind that the epithelium of the rabbit lens represents about 5% of the mass of the lens and contains probably several times as much glutathione as the average value for the whole lens. If only the epithelium and a thin ring of young lens fibers, which also have a very high glutathione level, were damaged by the deviation from isotonicity, while the remaining parts of the lens would resist the injury, the unequal distribution between lens and environment could be explained.

Wachtl and Kinsey [63] investigated the utilization of glucose and two other hexoses—galactose and fructose—and of xylose by rabbit lens in tissue culture and the effect of these four sugars on the mitotic activity of the epithelial cells of the lens. The lenses were cultured in two modifications of the standard lens-culture medium previously described by the authors. In one modification 0.8% of rabbit plasma was added to the

medium. In the second modification the nucleosides and α-ketoglutaric acid were omitted in the standard medium and glutamine and TPN were added. This second medium was able to support a normal level of mitosis in cultured lenses for nine hours. The lenses were cultured at 37 C at pH 7.55 in an atmosphere of 88% nitrogen, 7% oxygen, and 5% CO_2. They utilized glucose at a rate dependent on concentration between 125 and 250 mg. % of substrate. The lactate-to-glucose ratio varied between 0.4 and 0.91. Galactose was utilized to a less extent and increased with the concentration in the same range. The lactate-carbohydrate ratio varied between 0.28 and 0.62. A still greater dependence on concentration was observed in presence of fructose. All of the fructose metabolized could be recovered as lactate when the concentration was 125 mg. %. At 250 mg. % the ratio of lactate to fructose declined. Xylose was used up at about the same rate as galactose but did not produce any lactate. When these sugars were added together with glucose, then glucose was utilized preferentially, the utilization of galactose and xylose not being significantly changed by glucose. The mitotic activity proved to be completely dependent upon the presence of glucose, and the other sugars could only support mitosis at a small fraction of the normal rate. There was no significant influence of these sugars when they were present in the medium together with glucose.

Delogu [66] studied the influence of galactose on survival of the lens cultures in vitro. Rabbit lenses kept in Ringer solution gained 4.2% in weight when the solution contained 1% galactose as compared with 3.3% when it contained 1.1% glucose.

Cinsa and co-workers [67] found the amide of nicotinic acid in the lens, whereas the aqueous contained the acid itself.

Heinrichs and Harris [68] investigated changes with age in the ability of the lens to respond to stress by measuring the recovery from the water and cation shift induced by refrigeration. These experiments were stimulated by previous observations that age of the lens alters the responses to high glucose level and to deprivation of glucose. The experiments were carried out on fresh intact rabbit lenses which were extracted with particular care taken not to touch the lens surface. Lenses varying in weight between 65 and more than 125 mg. were used for the experiment, the weight of the lens being taken as indicative of the age of the animal. They were refrigerated for 40 to 44 hours at 0 C and then transferred to a water bath of 37 C, incubated for an additional six hours, and dried for 48 hours at 105 C; the dried material was weighed and dry-ashed with a drop of concentrated sulfuric acid. K and Na were then determined by flame photometry. No significant differences in the initial content in sodium were found in the size range studied. When recovery was carried out in a modified Tyrode solution, the extent to which the lenses recovered in per cent of the original concentration of cations was found to be inversely proportional to the size of the lens. Thus, 4- to 6-week-old albino rabbits recovered completely, whereas lenses from 4- to 12-month-old albino rabbits, only 50%. When lenses were incubated at a higher concentration of glucose, 500 mg/100 cc., no exact correlation between the effect of high glucose concentrations and the age of the lens was observed, although cation recovery was changed in every lens, in the sense that the recovery of K concentration was reduced while that of Na was not. These observations indicate a dissociation of the movement of the two cations. When lenses were incubated in absence of any glucose in the surrounding medium, lenses of 4- to 6-week-old rabbits recovered to a certain extent but not those of older animals. Here, again, there was a disparity between movements of K and Na. A similar dissociation was observed when lenses of animals of different ages were incubated at 37 C without prior refrigeration. In presence of glucose the concentration of Na remained constant during 40 hours of incubation but increased in

absence of glucose while the concentration of K in both instances tended to rise. When refrigerated lenses were incubated in absence of glucose and under anaerobic conditions, no recovery of cations occurred at all. The authors believe that these results are due to a reduced metabolic level in older lenses. They discuss the possibility that younger lenses possess an alternative metabolic pathway which makes it possible for them to retain their cations when incubated in absence of glucose. They also interpret their results as indicating a separate pump for potassium and sodium, which appear not to be completely linked to each other. They assume that the unlinked portion of potassium movements during recovery does not require glucose in younger lenses, whereas the independent movement of sodium is completely dependent upon an exogenous glucose supply.

Farkas and Paterson [69] reviewed previous experiments on the influence of insulin on glucose uptake by lenses in vitro and report new experiments which were undertaken to explain the fact that insulin decreases the glucose uptake when injected in vivo but does not decrease it when added to the lens in vitro. When all viscera except the liver were removed from rabbits, the insulin effect on the lens after injection in vivo still was demonstrable, and at the same time it was found that the uptake of glucose by the lens in these animals was appreciably increased even without insulin. This suggests that the effect of in vivo injected insulin on the glucose uptake by the lens may be due to the effect of the hormone on the liver, which leads to the production of a substance which influences the glucose uptake by the lens. An inhibitor of the glucose uptake may be present in other internal organs.

Dische and associates [70] continued their experiments on the changes in the chemistry of lens proteins and in protein synthesis during the development of galactose cataract. They showed that the inhibition of the synthesis of soluble proteins is already found during the six-day period after the start of galactose feeding. As the inhibition varies between 60% and 100%, these data indicate that the inhibition appears immediately or almost immediately after the level of galactose in the blood decreases. The increase in the protein which precipitates after oxidation of the lens homogenate with $K_3Fe(CN)_6$ is also demonstrable to the full extent after six days. The fractionation of the lens proteins in normal and galactose-fed rats has shown that this protein, precipitable after oxidation, is present in the β-crystallin fraction and not in the α-crystallin fraction. The presence of α-crystallin exerted an inhibitory effect on the precipitation of this protein. Such a precipitable fraction was also found in the β-crystallin prepared from lenses of 2-week-old rabbits, but it was absent in the β-crystallin prepared from 6-month-old rabbits and absent in the β-crystallin of 4-month-old calves. The authors assume, therefore, that the amount of this protein, which is only demonstrable in lenses of young animals, is related to the rate of protein synthesis in the lens, as expressed by the amount of protein synthesized per unit volume. They also suggest that the cataractous changes due to galactose feeding are the result of the discrepancy between the rate of synthesis of soluble proteins and the insoluble albuminoid which is not significantly affected by galactose. This discrepancy, in combination with changes of the lens volume which were found to take place, leads to a dilution of the soluble proteins inside the fibers and to an increase of the concentration of albuminoid. If the latter is deposited on the surface of fibers, this increase in the concentration may lead to metabolic disturbances by influencing the exchange of nutrients.

Ogino and his associate [71] reported interesting findings on the cataractogenic properties of various quinoid substances. This investigation represents a follow-up of previous findings of these authors on the excretion of naphthoquinones in the urines of naphthaline-treated rabbits and on the enhancement of the effect of galactose feedings by tyrosine and its oxidized metabolic products. In a first series of experiments

the authors showed that ether extracts of patients with senile cataracts (eight cases) injected into guinea pigs weighing about 300 gm., which were kept on a ascorbic acid-deficient diet for five days, produced cataractous changes after a few days, while controls injected with identical ether extracts from noncataractous ophthalmological cases did not have such an effect. The substance which was extracted with ether from the urine acidified with HCl was found to be not precipitated by lead acetate and lead subacetate but was precipitated by the Hopkins-Cole reagent ($HgSO_4$-H_2SO_4). The authors isolated two cataractogenic substances from the urine of cataract patients by ether extraction, purification with lead acetate, and precipitation with the Hopkins-Cole reagent, which by paper chromatography with *n*-butanol acetic acid water and by melting points of crystalline substances were identified as anthranilic and 5-hydroxylanthranilic acid; 25 mg. of the first and 10 mg. of the second compound were obtained from 12 liters of urine. The authors then prepared the quinonimine-carboxylic acid from 5-hydroxylanthranilic acid by oxidation with bromine and showed that injection of this substance obtained as oxidation product of 15 to 20 mg. of hydroxylanthranilic acid produced cataracts in scorbutic guinea pigs. The isolation procedure used for the anthranilic and hydroxyanthranilic acid carried out on urine from noncataractous patients did not yield any of these two compounds. The authors suggest, therefore, that disorders of the tryptophan metabolism are an important pathogenic factor in senile cataracts.

In a second series of experiments Ogino and associate[72] report that they were able to produce cataracts in scorbutic guinea pigs by injections of 10 mg. of 2,4-dinitrophenol, while control animals, which, in addition to the ascorbic acid-deficient diet, received 2 mg. of ascorbic acid daily, did not develop any cataracts. It is unfortunately not clear from the paper whether the 10 mg. of dinitrophenol was given only once or daily. As dinitrophenol is oxidized in the animal body to numerous oxidation products, a series of such intermediates was also tested for their cataractogenic properties. These were found in *p*-nitrophenol, 2-nitro-4-aminophenol, 2-amino-4-nitrophenol, and 4-nitro-6-cyclohexylphenol. It appears, therefore, that the para position between the hydroxyl and a nitro or amino group is essential for the cataractogenic properties. In an attempt to isolate the cataractogenic agent which possibly was formed from dinitrophenol and its oxidation products, the urine was extracted exhaustively with ether and precipitated first with lead acetate and then with the Hopkins-Cole reagent, which was found to precipitate the cataractogenic substance. The authors isolated in crystalline form a black-brown substance which on the basis of its ultraviolet absorption and its physical and chemical reactions was identified tentatively as 2-amino-*p*-quinonimine prepared synthetically by bromine oxidation from 2,4-diamino phenol. This substance was found to be excreted in combination with acetic acid in the urine of animals fed 2,4-dinitrophenol.

In a third paper the authors[73] report the histological findings on cataractous lenses obtained by feeding naphthalene, dinitrophenol, and quinonimine-carboxylic acid, as well as galactose plus tyrosine, to guinea pigs on ascorbic acid-deficient diet. The investigation was also extended to rats given alloxan, which received tyrosine daily when they became diabetic. The naphthalene cataracts were produced in male albino rabbits of 2000 gm. weight eight or more days after the beginning of the feeding. The diabetic cataracts were investigated on 100 gm. albino rats which were first given 35 mg. of alloxan intravenously and became diabetic two days later. They received 100 mg. of tyrosine daily, starting on the fifth day of the experiment and 90% of the animals showed cataractous changes two weeks later. The other forms of cataracts were studied in guinea pigs on ascorbic acid-deficient diet. In all cataracts similar changes were observed and reported, which

consisted in vascularization predominantly in the equatorial region and degenerative changes and swelling of epithelium and lens fibers. The findings of Ogino and associates suggest that quinones, as strongly oxidizing agents, may induce cataractous changes when the reducing substances of the lens, namely, glutathione and ascorbic acid, are totally or partially removed from the lens. It is clear, therefore, that these substances cannot be the primary cause of cataract, but they may represent a complimentary factor necessary for the formation of certain types of cataract. It must be noted, however, that the role of these substances as cataractogenic agents is at present difficult to assess because of the importance of the concentration factor. The amounts of substances necessary to produce experimental cataracts apparently are of an order which is not comparable in the least degree with the concentrations which occur in vivo.

Van Heyningen and Pirie [74] took up an investigation started in 1953 on the effect of x-ray irradiation of rabbit lenses on their hexokinase activities. These experiments were suggested by the work of Nordmann and Mandel (1955) and Mandel and Schmidt (1956), who reported a sharp decrease in the activity of these enzymes two to four days after irradiation. The right eye received a single dose of x-rays of 1400 r. The left eye served as a control. Two series of experiments were carried out, one on 17- to 23-week-old animals which were killed 17 to 37 weeks after irradiation, when the lens was partially or completely opaque, and another on rabbits 6 to 8 weeks old, which were killed two to seven days after irradiation. The lenses were ground with sand and mixed with an equal volume of a phosphate buffer of pH 7.8 which contained 0.15 M of sodium fluoride. In addition, $MgCl_2$, 0.015 M; KCl, 0.125 M, and glucose, 1 mg. per milliliter, were added to the fluid. One sample of a lens suspension was immediately deproteinized; the other was incubated at 37 C for four hours, after which the reaction was stopped by deproteinization with TCA. The activity of hexokinase was determined by determining the amount of glucose which disappeared. In the second series of experiments a purified preparation of phospho hexokinase was added to the incubation fluid to assure a complete removal of hexose monophosphate by phosphorylation and, therefore, avoid the objection that the accumulation product of hexokinase activity influenced the enzyme. In these experiments, with much younger animals, which showed a much higher hexokinase activity per lens, it was possible to use a greater dilution of lens and to measure the disappearance of glucose after a period of one and one-half hours. The amount of hexokinase was chosen so that it was at least five times the amount needed to remove the hexose monophosphate as fast as it was formed. The glucose was measured by the method of Nelson (1944). The irradiated eyes did not show any differences in their hexokinase activity in comparison with the controls until the lenses were almost completely opaque. Even in the latter case, the activity of the enzyme remained unchanged in some of the lenses, while in others it dropped between 25% and 75%. In these cases the decrease in hexokinase activity also showed up in a higher level of glucose in the lens. The authors discuss possible reasons for the discrepancy between their results and those of Nordmann and Mandel.

Flanders and Pirie [75] examined the effect of higher oxygen tension in the atmosphere on the susceptibility of the lens to x-rays. As a measure of this susceptibility they used changes in the glutathione content of rabbit lenses. The experiments were carried out in such a way that the experimental rabbits were exposed to an atmosphere of 95% oxygen plus 5% CO_2, while the controls remained in a normal atmosphere. To accelerate the exchange of oxygen in the aqueous, the pressure of the gas was raised to 2 atmospheres for 15 minutes and then reduced to atmospheric pressure 5 minutes before irradiation. Irradiated eyes were examined with the slit lamp and ophthalmoscope at weekly intervals and the

the animals were killed when the lens opacities, according to previous experience, could be assumed to be associated with a loss of glutathione of about one-half or two-thirds of the original amount. The eyes were dissected out, and the lenses were removed, weighed, and precipitated with 10% TCA. The glutathione was determined in the supernatants. The animals irradiated in oxygen and the animals irradiated in air were from the same litter. In seven such littermates the dose of x-ray necessary to produce an equal effect in the two littermates was found to be on the average 24% higher in the air than in oxygen of 1 atmosphere pressure. The authors point out that the lens was at 80% of the maximum sensitivity when the oxygen tension was about 15 mm. Hg. This agrees with what was found about the relation of sensitivity and oxygen tension in the case of the ascites tumor of the mouse.

Vitreous

Varga and Gergely [76] estimated the length of molecules of hyaluronic acid prepared by electrophoresis from cattle vitreous using flow birefringence measurements. The eyes were obtained from 1- to 2-year-old steers one hour after killing and were stored until used at 5 C. Posterior portions of the vitreous body were dialyzed for 24 hours at 5 C against 4 liters of phosphate buffer pH 7 and the material was centrifuged in the preparative ultracentrifuge at 80,000 g. The supernatant was concentrated to one-eighth of its original volume by pervaporation at 4 C and again dialyzed. Hyaluronic acid solution, 3.5 ml., was removed from the Tiselius apparatus and subjected to a reelectrophoresis so that only one peak was found. The analysis of nitrogen, glucosamine, glucuronic acid, and sulfur showed that no more than 3.5% protein and 1% chondroitin sulfate were present in the preparation. The calculation of the length of the molecule at different velocity gradients changed from lower to higher gradients from 1550 to 1750 A., indicating only a low degree of polydispersity. The authors, however, point out that a higher degree of polydispersity could be masked by the flexibility of the chain of hyaluronic acid, which at higher shear could gain in length. The authors compare their results with those obtained with light-scattering and sedimentation and diffusion measurements. The higher value of 3000 A., in their opinion, is not correct and is due to the polydispersity of preparations of hyaluronic acid used for the measurements. The lower value of 1000 A., obtained by sedimentation and diffusion, may be due to the fact that preparations which were measured in this way were obtained by solvent fractionation procedures, which lead to a certain degradation of the material. They believe, therefore, that their values are most likely to be the correct ones.

Laurent [77] summarized his investigations on the physicochemical properties of hyaluronic acid, which were carried out between 1951 and 1957, using light-scattering viscosity and streaming dielectricity as well as x-ray diffraction measurements.

To determine whether the methods of isolation of hyaluronic acid are responsible for the great variations found in molecular sizes, Laurent [78] used hyaluronic acid from five different tissues, namely, umbilical cord, vitreous body, synovia, skin, and cock's comb, prepared by the method of Scott, which is based on removal of protein by papain digestion and precipitation of the mucopolysaccharide with cetylpyridinium chloride. It was found that the hyaluronic acid from the vitreous body had a molecular weight of 3×10^6, which was the lowest value of all five, in agreement with observations of Blix and Snellman (1945). To test the influence of methods of separation, hyaluronic acid from vitreous body was prepared in two different ways, namely, by the Scott procedure and one based on precipitation of proteins at high and low ionic strengths. Comparable molecular weights were found in both preparations, although the preparation obtained without enzyme treatment represented a multicomponent

system characterized by the presence of a large-particle fraction, which is probably due to the formation of aggregation between small amounts of protein and hyaluronic acid molecules. Papain had no depolymerizing effect on hyaluronic acid in the author's experiments, but it reduced viscosity. The experiments on hyaluronic acid from umbilical cord, prepared according to the Scott method and two other methods, showed molecular weights of the same magnitude but widely different limiting viscosity numbers. The author concludes that these molecules differ by their solvent-draining capacity, owing to weak intramolecular bonds between the different parts of the polysaccharide chain. These linkages should not influence the shape of the molecule, however.

An amorphous x-ray diffractogram of hyaluronic acid was evaluated by Laurent [79] with the aid of a Fourier analysis.

In x-ray diffraction studies Laurent [80] investigated whether hyaluronic acid in water induces a greater order in the water lattice of the whole bulk of the solution comparable to the influence of lowering the temperature, but no changes of the pure water diffractions comparable to temperature decrease were observed in 2% solutions of hyaluronic acid.

To study the problem of sulfation of hyaluronic acid, the carboxyl groups of the latter were coupled with cetylpyridinium ions by Laurent.[81] The compound formed was insoluble in water but soluble in lower alcohols. Its light scattering and viscosity were studied in methanol. In spite of the coupling of the polysaccharide to a large polyelectrolyte ion, the molecule was found more coiled than in aqueous solution, which shows the importance of water and charged groups on the molecular chain for its configurational state.

Sugiura [82] found by electron microscopy, x-ray diffraction, and infrared spectroscopy that the fine fibrils of the vitreous are primitive collagen fibrils.

Yamada [83] reports that hyaluronidase injected into the vitreous in vivo or in vitro causes degeneration of vitreous fibers which can be observed by phase microscopy.

Schweer and Pook [84] applied the biuret reaction used by Ditterbrandt for protein determinations in the cerebrospinal fluid and by Weichselbaum for determinations of the plasma proteins, for the determinations of proteins in the vitreous. When various dilutions of plasma proteins were used as standards, the protein concentrations found by these methods in the vitreous were about 50% higher than those obtained by micro-Kjeldahl determinations or proteins precipitated with TCA. This is due to the fact that one-third of the total nitrogen of the vitreous was found to be present as nonprotein nitrogen, which is not precipitated by TCA. Correct values for vitreous proteins can be obtained by this method if dilutions of vitreous are used as standard, as apparently the ratio of protein to nonprotein nitrogen in individual samples of vitreous appears to be fairly constant. The determinations on 30 cattle eyes gave a value of 39.2 ± 3.7 mg. in 100 cc. of vitreous.

Schweer and Pook [85] determined the distribution of proteins among different parts of the vitreous body and the dependence of over-all protein concentrations of the vitreous on the age of the animal. The aim of this investigation was to determine whether the concentration of the proteins in the vitreous shows a pattern similar to that of hyaluronic acid and whether the amounts of hexosamine combined with protein can have a significant effect on the determination of hyaluronic acid of the vitreous from its glucosamine content. The determination of proteins was carried out by the above-mentioned procedure based on the biuret reaction, which the authors modified for determination in the vitreous. Three age groups of animals were used, namely, calves up to 3 months old, 1- to 6-year-old animals, and animals 7 or more years old. The main value from 10 determinations for the first group was 42.5 ± 3.26 mg. %, as compared with 37.6 ± 2.97 and 37.3 ± 2.25 mg. % for the two other groups. The

vitreous of young animals showed slightly but significantly increased values, whereas the glucosamine content was previously shown to be much lower in these animals than in older ones. On the other hand, the middle age group did not differ from older animals, although it showed much higher hyaluronic acid values. From their protein values the authors calculated the amount of hexosamine bound to proteins, under the assumption that it is identical with that in serum proteins. The validity of this assumption appears very doubtful, but under any circumstances the amount of glucosamine combined with protein appears negligible in comparison with that present in form of hyaluronic acid. For the examination of the distribution of proteins the vitreous body was divided into a posterior part, a peripheral anterior part, and a central anterior part. The first part showed somewhat higher values than the two others, in agreement with differences in distribution of hyaluronic acid.

Green and associates [86] investigated the distribution of the bicarbonate in various parts of the vitreous and compared its concentration in this part of the eye with that in the aqueous and plasma. They found that the over-all concentration in the vitreous of the rabbit eye is 25.3 mμ per liter, as compared with 19.4 mμ in the plasma, 30.1 mμ in the anterior chamber, and 36.0 mμ in the posterior chamber. The concentration was significantly higher in the anterior part of the vitreous, adjacent to the posterior surface of the lens and the ciliary body, than in the posterior part of the vitreous. Only a small difference was found between the central and peripheral parts of this posterior part. This indicates that either the ciliary processes or the lens are the source of bicarbonate. However, the authors point out that the gradient observed between the different parts of the vitreous is such that it does not permit firm conclusions as to the source of the bicarbonate in the vitreous.

In a second report, Green and Mann [87] made an attempt to resolve the discrepancy between their findings that the level of bicarbonate in the rabbit vitreous is higher than in arterial plasma and the findings of Auricchio, who found lower values than in the plasma. The cause of this difference was traced to the difference in the time of killing the animal. When the eyes are enucleated not later than two minutes after the killing of the animal by air embolism and the eye is immediately frozen before the determination of bicarbonate is carried out, then the bicarbonate level is found to be identical with that found in in vivo experiments when the vitreous is taken out by cannulation through the sclera.

Cristiansson [88] in three series of experiments thoroughly investigated the influence of scurvy in guinea pigs on the viscosity and composition of the vitreous In the first series growing guinea pigs of both sexes weighing 200 to 300 gm. were kept on a scorbutic diet, according to Kirk and Tressler, in groups of four to six animals each. At the end of the second week the animals started to lose weight and to show symptoms of the disease. Vitreous collected from five animals was pooled, and control experiments, in which the weight of the vitreous of the left and right eyes was compared, indicated that no major error due to differences in collected amounts was made. For the analysis, the vitreous bodies were filtered through three successive sintered glass filters of the Jena type with the mean pore diameters decreasing from 15-14μ to 1μ. The viscosity measurements and analysis were carried out on the filtrates. This filtration was carried out either on the native vitreous or after treatment with hyaluronidase. The vitreous was protected against depolymerization by the addition of 250μg. per milliliter of sodium diethyldithiocarbonate. Viscosity was measured with a modified Ostwald viscometer of the semimicro type. The viscosity-reducing power of the vitreous and aqueous was measured on a hyaluronic acid preparation from human umbilical cord. Ascorbic acid was determined in aqueous humor with Tilmann's reagent after deproteinization with 10%

metaphosphoric acid. Glucosamine was determined by the Elson-Morgan procedure as modified by Blix and Sundblad. The glucoronic acid was determined by the carbazole reaction; hydroxyproline in the whole vitreous, by the method of Neuman and Logan (1950) after 18 hours of hydrolysis at 100 C. Viscosity determinations on the vitreous freed from the residual protein by ultrafiltration was found to be significantly lower in the vitreous of scorbutic animals after 10 days of scorbutic diet. These findings were obtained when groups of normal animals were compared with those kept on scorbutic diet or when one eye of an animal was removed for determination before the start of the scorbutic diet and the other was removed at various time intervals of the scorbutic diet and its viscosity was compared with that of the other eye. The lower viscosity showed up also during filtration, which was appreciably faster in the case of scorbutic animals. Treatment with hyaluronidase depressed viscosity in normal and experimental vitreous to the same level. Glucosamine determinations on the vitreous, pretreated with hyaluronidase and passed through the three filters, showed a significant decrease in the eyes of the scorbutic animals as compared with the other eyes of the same animals removed before the start of scorbutic diet. This was not the case, however, if the native, not pretreated, vitreous was used for determination. In this case, the eye removed from the scorbutic animal showed significantly higher glucosamine content than the previously removed eye. This difference became excessive 21 days after the start of the diet. The authors suggest that this difference between the results on the native and the depolymerized vitreous is due to the fact that hyaluronic acid from animals with scurvy is less easily adsorbed on filters than that of normal eyes. The possibility that there are differences in the relation of hyaluronic acid to the residual protein removed by ultrafiltration apparently is not considered by the author. The determinations of glucuronic acid showed a decrease in the vitreous body in a group of six animals 19 days on scorbutic diet, as compared with a group of five normal animals. There were no differences in the residual proteins determined by hydroxyproline determinations. The water content of the scorbutic vitreous was on the average 4% higher than that of the normal vitreous, and this difference was shown to be statistically significant. When scorbutic animals were injected subcutaneously with large quantities of ascorbic acid 13 days after scorbutic diet, when a significant decline in glucosamine in the vitreous took place, the glucosamine concentration and water content of the vitreous returned to about normal after a treatment of seven days. Ascorbic acid, glucosamine, and proteins were also determined in the aqueous of normal and scorbutic animals. In agreement with previous findings of other authors, there was a sharp decrease of ascorbic acid after 10 days of scorbutic diet to a mean value of 1.3 mg/100 gm. of aqueous. On the other hand, the glucosamine in the aqueous, which in the normal was found to be $11.9 \pm 0.2 \mu$g. per gram, went up in scorbutic animals after 16 to 19 days to $7.5 \pm 1.1 \mu$g., which, in spite of low values which make the analysis difficult, was found to be significant with ($P < 0.01$). There was no increase of protein in the aqueous of scorbutic animals, which suggests that the increase in glucosamine in the aqueous is not due to a lowering of the blood-aqueous barrier in scorbutic animals but rather to an increase in the diffusion of hyaluronic acid from the vitreous due to depolymerization. It is of particular interest, however, that the vitreous of the scorbutic animals did not show any depolymerizing activity of hyaluronic acid, which was found in normal vitreous, and which is obviously due to the ascorbic acid. The author interprets these results as indicating that no decrease in the production of hyaluronic acid in the ciliary epithelium takes place in scurvy but that the depolymerization of the mucopolysaccharide leads to a change of its interaction with the proteins of the vitreous, and he believes that this situation is analogous to that found in

connective tissue of scorbutic animals. This analogy extends also to the higher water content in scurvy.

In a second series of experiments, Cristiansson [89] determined changes in the dry weight and adsorptive capacity of the organic constituents of the vitreous for water in normal and scorbutic animals. The determinations were carried out on vitreous passed through a glass filter with 15 to 14 mμ average pore size. The removal of the vitreous was carried out in such a way that no vitreous was lost on the uveal tissue. The determination of the dry weight and the adsorption were carried out in the following way. For measurements of adsorption one eye of the animal was removed before the start of the scorbutic diet and the other one, 20 days later. The two filtrates were divided into equal volumes on weighed watch glasses dried in a vacuum oven, as mentioned above, and, after the determination of the dry weight, the watch glasses were placed in well-sealed glass jars with different relative humidities. These were regulated with the aid of concentrated sulfuric acid. The temperature was maintained at 22 C, and the equilibration was followed for seven days. The water-uptake humidity curves of the normal and scorbutic eyes were then compared. Those from scorbutic eyes showed a significantly lower adsorption in the first two segments of the adsorption curve, namely, that representing the Langmuir adsorption, in which monomolecular layer of water is formed on the surface of the polymer molecules, and that in which a linear relationship exists between adsorbed water and vapor pressure. The dry weight was also significantly lower in the scorbutic vitreous. As there is no increase in the intraocular pressure in scurvy in spite of the increase in the volume of the vitreous and a decrease in dry weight and hyaluronic acid, the author believes that the hyaluronic acid secreted in scurvy is incompletely polymerized and differs in its polar groups which are responsible for the interaction between the mucopolysaccharides and the proteins of the vitreous. This leads to a disturbance of the gel structure of the vitreous and liberation of hyaluronic acid, which is not incorporated in the vitreous gel. He does not believe that an increase in the osmotic pressure of the vitreous is operative in the greater hydration; the latter rather is the result of an increase in the solubility of the hyaluronic acid which is not incorporated in the gel. This concept does not appear sufficiently elaborated.

In a third series of experiments,[90] Cristiansson determined the influence of scurvy on the distribution of sodium and *o*-phosphate between the blood plasma, the aqueous, and the vitreous of guinea pigs. He also investigated the rate of exchange of Na^{24} and P^{32} administered intraperitoneally between the blood and the two ocular fluids in normal and scorbutic animals. The number of experiments was small; the animals weighed 400 gm., and the scorbutic diet was continued for 17 to 18 days in experiments with sodium and 19 to 23 days in experiments with phosphate. The control animals received the same diet, but the diet was supplemented by parental administration of ascorbic acid. The ratio of concentration of Na in aqueous to that in blood was found to be 1.02 and not be affected in scurvy. In the vitreous it was somewhat smaller than in the plasma, and it was identical in normal and scorbutic animals. No influence of scurvy was found in the exchange of Na^{24}. The level of *o*-phosphate in blood plasma and aqueous showed no significant differences under the influence of the scorbutic diet. In the scorbutic vitreous body, however, the level was only 8.8 ± 0.5 as compared with 10.7 ± 0.5 in the normal. The author cannot decide at present whether this decrease is due to a slowing of the rate of entry, or to an increased consumption of ortho phosphate. The activity curve of P^{32} showed a significant depression in the vitreous from scorbutic animals. The author discusses the possibility that an increased Donnan effect due to the polar groups of the hyaluronic acid, which is no longer bound to residual proteins, might be involved in

the decrease in the inorganic phosphate in the vitreous body in scurvy.

Retina

Lewis [91] determined the absorption spectrum and the maximum density of rhodopsin in the retina of the albino rat in vivo by a method similar in principle to that described by Rushton (1956). The measurements of the absorption of reflected light were checked by measurements of the light transmitted through the retina of eyes removed from the body. The measurements were carried out with an instrument very similar to that of Rushton, except for the apparatus used to produce light flickering between red and other lights, which is described in detail. The rats were males weighing 200 to 300 gm. anesthetized with 7.5 ml. of 25% urethan per kilogram. They were atropinized and kept warm on a heated box. For experiments on isolated eyes the rats were kept in the dark overnight and after atropinization killed by urethan and the head was drained of blood. The experiments were carried out during a period of no more than 15 to 20 minutes, to prevent clouding of the lens. Difference spectra were obtained by measurements of at least 6 and, in particularly stable preparations, of 12 wavelengths. In isolated eyes, because of the threat of clouding, measurements were carried out at only three or four wavelengths. The two kinds of difference spectra agreed fairly well, and the ratio of the density measured by reflection to that measured by transmission was 1.87, indicating that 7% of the light was scattered by the ocular media. The difference spectra measured by the two kinds of light were in reasonable agreement, except at wavelengths of 441 mμ, at which light is strongly scattered in the eye media but poorly reflected from the fundus. More than 50% of green light was found to be absorbed in the retina. The maximum rhodopsin density calculated from the mean density of rhodopsin extracted from the dark-adapted rat retinas and corrected for the orientation of rhodopsin molecules in the rods, the funneling of light from an inner to an outer segment, and the difference in the distribution of rhodopsin in various parts of the retina yielded a value which was only two-thirds of that experimentally found, a discrepancy found also by other authors in retinas from other species.

In another series of experiments Lewis [92] determined the regeneration of rhodopsin in albino rats, with particular emphasis on the effect of light and temperature on this process. Only a small number of experiments were completely successful because of the difficulty of keeping the animals in the dark and covered for a period of at least five hours necessary for the experiments. The regeneration was measured after complete bleaching of the retina with white light by comparing the light absorption in the reflected light of 488 mμ with that of 618 mμ. To check on fluctuation of the blood supply of the choroid, control experiments with light of 575 mμ strongly absorbed by hemoglobin were also carried out. Regeneration was found to be linear for some three hours at a rate which varied between 0.08 and 0.13 log units per hour. The maximum rhodopsin density reached also 0.38 density units. The data on the rate of regeneration found by the author is not in agreement with previous results of Tanseley on the same animal. Control experiments of the author on anesthetized animals showed that this difference is not due to anesthesia. That light has no influence on the rate of regeneration was shown by comparing the density of rhodopsin measured at the equilibrium by bleaching and regeneration with light of different intensities. In this experiment the bleaching lights were bands of wavelengths 517 and 416 mμ. A drop of 10 degrees in the temperature of the animal caused a drop of the regeneration rate of no more than 10% to 20%. The author concludes, therefore, that this rate is not limited by any of the balanced chemical reactions involved in bleaching of rhodopsin and regeneration but

by the rate of diffusion from the blood of an essential ingredient.

Weale [93] reported experiments on cats on changes in fundus reflectively in living eyes and carried out also similar determinations on frog eyes, using a previously described photometric method. The experiments were carried out on anesthetized descerebrated animals and showed in both animals very rapid increase in the density of visual pigment after the light was shut off, with a maximum reached after about one minute. The time-density curve of this effect is very similar to that found by Granit with photopic dominators with his electrophysiological procedures. These observations suggested the desirability of very rapid spectral recording of density changes in the living eye, and a new spectrophotographic method, therefore, was developed for this purpose, which is described in detail. With this method the photosensitive material was analyzed in living cat and rabbit eyes. A secondary change was observed in these experiments, spectrally similar to *m*-rhodopsin. Thus, this is the first demonstration of the presence of this intermediate in the bleaching process in vivo.

Linschitz and associates [94] made an attempt to demonstrate the transient intermediates in the initial processes of rhodopsin bleaching which are involved in the electrical retinal response. Rod fragments of rabbit retinas were isolated according to the usual procedure of St. George. Rhodopsin solutions were irradiated with light flashes of 15 millisecond duration, and the transmission of the solution was measured in the dark 1, 2, 14, and 40 milliseconds and 1 hour after the flash. The experiments showed the formation of a transient intermediate with an absorption maximum at 485 mμ and lower transmission after the first millisecond and an increase in transmission in the following millisecond. A third component developed during the following 40 milliseconds. The authors point out that because of the time factor only the first two intermediates can be responsible for the electrical light changes in the eye.

Albrecht [95] made an attempt to determine the C-terminal and N-terminal amino acids of rhodopsin. The latter was prepared in the usual way from cattle retinas. Its purity was characterized by its 400:500 ratio of 0.22 to 0.26 and of the 278:500 ratio of 2. The N-terminal groups were determined by the method of Senger and of Levy and Li. The chromatographic procedure of Blackburn was used for determination and identification of the dinitrophenol amino acids. C-terminal groups were determined with carboxypeptidase (Worthington Biochemical Corporation), according to the method of Neurath and Schwert. Dichlorophenol in 10^{-5} mμ per milliliter was added to inhibit other proteolytic activities. Small-molecular-weight peptides and amino acids in rhodopsin were prepared by chromatography on Dowex 50 resin in the hydrogen form and eluted from the resin with 5N NH_4OH. They were identified by one-dimensional chromatography with butanol acetic acid water. The purification of rhodopsin was carried out by chromatography on the mixed-bed column, according to Dintzis (1952). The rhodopsin was eluted with a digitonin solution. This procedure avoided bleaching of rhodopsin. The small-molecular-weight peptides which contaminated rhodopsin were not absorbed on the column. The purified rhodopsin was treated with fluorodinitrophenol at pH 8 for 12 hours with the exclusion of white light. DNP rhodopsin precipitated as a yellow powder; this was washed with water, ethanol, and ether and dried in air. All digitonin was thus removed. After hydrolysis for four and eight hours in 5.7 N HCl at 110 C in sealed tubes, no other DNP amino acids than ε-DNP-lysine were found. This purified rhodopsin, therefore, does not contain any N-terminal amino acids. The C-terminal analysis of purified rhodopsin was carried out in bleached and unbleached form by incubation with carboxypeptidase for various lengths of time. Digitonin used for

dissolving rhodopsin was found not to interfere with the enzyme. No amino acids were liberated by this treatment even after an incubation of nine hours and a substrate enzyme ratio of 10:1. When the nonpurified rhodopsin was passed through a Dowex 50 ion-exchange resin in the hydrogen form and eluted with ammonia, paper chromatography of the eluate showed two distinct ninhydrin-positive spots with R_f's similar to those of lysine and glycine. The latter spot is more intense. Elution of both spots followed by hydrolysis in 5.7 N HCl at 100 C for 24 hours indicated the presence of lysine, serine, glycine, alanine, and glutamic acid. The determination of the N-terminal amino acids in both these peptides showed that they contain glycine as the N-terminal acid. The molar ratio of glycine present in rhodopsin to rhodopsin itself was about 1:1. Treatment of the nonpurified rhodopsin with fluoronitrobenzene failed to reveal the presence of any N-terminal amino acids other than glycine. The author concludes, therefore, that rhodopsin is probably combined with a polypeptide by weak chemical bonds, as they cannot be removed by even drastic preextraction of retinal tissue with aqueous solvents or by exhaustive dialysis. The peptides are present in a quantity roughly equimolar with rhodopsin and they may have particular significance. The author points out that the results concerning the absence of C-terminal groups must be regarded as preliminary as long as no more reliable purely chemical methods of determination of C-terminal groups can be applied to rhodopsin.

Albrecht [96] prepared acetylrhodopsin from cattle rhodopsin purified by passage through columns of mixed ion-exchange resins by the procedure mentioned in the preceding paper and acetylated it by the method of Fraenkei-Conrat (1949). The free amino groups of acetylrhodopsin were determined by the ninhydrin method, with use of alanine as standard. Thirteen such groups were found in denatured rhodopsin, of which 74% became acetylated. Acetylation had no significant effect on the absorption spectrum of rhodopsin. The introduction of acetyl groups on the protein apparently does not disturb significantly the nature of the opsin-retinene relationship. However, the acetylated opsin obtained by bleaching of acetylated rhodopsin was unable to resynthesize rhodopsin in presence of neo-b retinene. While the opsin from rhodopsin at pH 6.5 regenerated 83%, acetylrhodopsin opsin regenerated only 5%, and this small amount might have originated in a contamination with unchanged rhodopsin. The acetylated pigment is also much less stable than rhodopsin itself, as storage at 4 C bleaches it completely in two weeks, while the original pigment remains unchanged. This loss in stability is probably due to a loosening of the structure of protein due to the elimination of hydrogen bonds involving amino groups. The elimination of the prosthetic group, which to a certain degree protects the protein against denaturation, leads then to complete denaturation and loss of the ability to resynthesize the pigment.

An impressive progress was made through the work of Wald and others in the comparative biochemistry of the visual pigments. This progress appears to contribute to the understanding of more general evolutionary problems. Wald [97] investigated the visual pigment of the sea lamprey (Petromyzon marinus). Older reports which indicated the predominance of vitamin A_2 over vitamin A_1 (4:1) in this species appeared contradicted by more recent investigations of Crescitelli, who found rhodopsin to be the pigment in the retinas of these animals. Wald resolved this contradiction by discovering that the visual pigments of the sea lamprey undergo a metamorphosis in which porphyropsin present in the youthful forms of the animal is replaced by rhodopsin in the sexually mature adult forms which migrate downstream to the ocean. Retinas of adult lampreys from Oyster River in Durham, N. H., were dissected out and immediately frozen. They appeared almost colorless. The outer layers of the rods were then prepared by

the usual fractionated centrifugation of ground retinas suspended in a phosphate buffer of pH 7 containing 40% sucrose. The outer layers accumulated in this procedure at the border line between the sucrose solution and pure phosphate buffer. Homogenization and fractionation processes were repeated three times. The outer layers so obtained were sedimented by centrifugation, washed with distilled water, hardened by 4% alum solution for 35 minutes, and washed again with water and phosphate buffer, and the visual pigment was finally extracted with 2% digitonin for one and three-quarters hours in the refrigerator. The downstream-migrating growing animals were taken from Lake Huron; the eyes were dark-adapted and removed under red light, and the whole fundi were subjected to the same homogenization and fractionation procedures. The absorption spectrum of the visual pigment of these animals proved to be a typical rhodopsin spectrum with an unusually prominent β-band maximum. After complete bleaching with nonisomerizing orange light, the typical mixed spectrum of all transretinene$_1$ and opsin, with absorption maximum about 373 mμ, was obtained. The reduction of this retinene with potassium borohydride gave the typical spectrum of vitamin A_1, with no suggestion of the presence of vitamin A_2. This was confirmed by the spectrum in the antimony chloride reaction. Comparable data from the mature upstream migrants showed the visual pigment to possess an absorption maximum at 518 mμ instead of 500 mμ in the immature animals, which is somewhat below the maximum of 522 mμ found for porphyropsin of freshwater fishes. After bleaching the typical absorption spectrum of transretinene$_2$ in the digitonin solution was obtained. After reduction an all trans-vitamin A_2 spectrum could be demonstrated. To test the homogeneity of the porphyropsin from immature sea lampreys, difference spectra obtained by bleaching by stages were obtained. The absorption curves showed a sharp common, or isosbestic, point at about 457 mμ, indicating complete homogeneity. This metamorphosis of the visual pigment of the sea lamprey is not associated with the metamorphosis of vitamin A in the liver, which during the whole life span remains vitamin A_1. The author discusses the implications of these findings for our concepts of the phylogenesis of the vertebrates. He stresses the analogy between the metamorphosis of the visual pigments in the sea lamprey and that in amphibia, like the bull frog tadpole, and in the New England spotted newt (Triturus viridescens). He also quotes his previous findings on euryhaline fishes, which possess in their growth phase mixtures of the two visual pigments, in which porphyropsin predominates, or porphyropsin alone. The second metamorphosis of the visual pigments in these animals does not represent the recapitulation of an early evolutionary transition, but is an adaptation of a life cycle that involves two environments. He adheres to the concept of the fresh-water origin of vertebrates and regards porphyropsin as their original visual pigment.

Following the report of Denton and Warren about the occurrence of a visual pigment with an absorption maximum at 480 mμ in a deep-sea fish living at a depth of about 280 fathoms, Munz [98] determined the absorption spectra of visual pigments of a series of deep-sea fishes living 280 to 380 fathoms beneath the surface. He confirmed the findings of Denton and Warren in the fish Argyropelecus affinis Garman, having a visual pigment with an absorption maximum at 478 mμ, which was found by partial bleaching to be homogeneous. The absorption of the product of bleaching had a maximum between 375 and 380 mμ, which decreased by 5 mμ when the bleaching was carried out in presence of hydroxylamine, which indicated its identity with retinene$_1$. In Sternoptyx obscura Garman, belonging to the same family of fish, a homogeneous visual pigment with an absorption maximum of 485 mμ was found, but in a deep-sea fish belonging to another family, Bathylapus wesethi, there were two

different visual pigments, one with an absorption maximum of 478 and one of 500 mμ, as in the rhodopsin of land animals. The ratio of the two pigments was found to be 4:1. The author stresses the identity of the prosthetic groups in these pigments with retinene$_1$, which indicates that the shift in the absorption maximum is due to differences in the protein, and believes that the term chrysopsin for these pigments is not justified and that they should be designated as rhodopsin and specified by their absorption maximum.

These findings of Munch stimulated an investigation by Wald and associates,[99] who determined the nature of the conjugate of the visual pigments of various marine fishes and made an attempt to correlate the position of the maximum absorption of the pigments and the depth of habitat of these animals. The summer range of the latter extended in various species between 10 and more than 200 fathoms. They found that the visual pigments of the lancet fish (Alepisarus ferox), which lives deeper than 200 fathoms and has a maximum of 480 mμ, and of cusk (Brosme brosme Müller), living between 10 and 100 fathoms, both bleached in orange light to a mixture of all transretinene$_1$ and opsin and can be regenerated in the dark when neo-b retinene is added to the bleaching product. On the other hand, in eight different species of these marine fishes the absorption maximum varied between 480 and 503 mμ, and in general there was a fairly good inverse correlation between the depth of habitat and the absorption maximum of the visual pigments. The authors, therefore, suggest that the pigments of these marine fishes, even if they significantly differ in their absorption maximum from the rhodopsin of vertebrates, should not be called chrysopsin but should be called rhodopsin with specification of the species from which they are derived. If obviously the variations in the optical properties are completely dependent on small variations in the nature of the opsins, the far-reaching adaptation of the absorption of the visual pigments of these fishes to the kind of light which predominates in their habitat appears to be of particular interest from the point of view of the study of evolutionary processes.

Wald and associates[100] investigated the visual pigments of the alligator (Alligator mississippiensis) because of the paucity of data about the visual systems of reptiles. Young animals 20 in. long were used. They were dark-adapted over night and decapitated, and the retinas and pigment layers were removed under red light. The tissues were dried by grinding with anhydrous sodium sulfate and extracted with chloroform. The extract was used for the antimony chloride reaction, which shows 2 maxima, at 620 and 660 mμ, respectively. This indicated that a mixture of vitamin A$_1$ and retinene$_1$ was liberated by chloroform from rhodopsin. From the optical densities at the two maxima and those of pure compounds, their amounts were computed to 0.171μg. of vitamin A and 0.525μg. of retinene per eye. In the pigmented layers as well as in liver only vitamin A$_1$ was present. Rhodopsin was then demonstrated in digitonin extracts prepared in the usual way. Direct absorption and difference spectra were taken which showed typical rhodopsin with an α-band absorption at maximum 500 mμ and β-band at 350 mμ. On bleaching a typical spectra of a mixture of all transretinene$_1$ and opsin was obtained. No red-sensitive pigments were found, as exposure for 20 minutes to deep red light showed an exceedingly slow bleaching with a maximum at about 500 mμ. The synthesis of rhodopsin was tested by adding 0.5 ml. of a solution of neo-b retinene to 0.4 ml. of a rhodopsin extract bleached by orange light. The mixture, incubated in the dark at about 25 C for 30 minutes, showed a rapid rise in the extinction of 500 mμ, which reached close to the maximum value in about 6 minutes. The difference spectrum was determined after addition of 0.1 ml. of hydroxylamine and appeared to be the typical retinene$_1$-opsin spectrum. Thus, the synthesis of rhodopsin appears to be so fast that it is impossible

to study the kinetic of the reaction at room temperature. Such experiments, therefore, were carried out at 4 C. The synthesis proved to be a bimolecular reaction, but in presence of a large excess of neo-b retinene it proceeded almost according to first-order kinetics, with a half time at 4 C at about 18 minutes, compared with about 10 to 20 seconds at 25 C. Experiments on the rate of dark-adaptation of the alligator eye showed a parallelism with the rate of synthesis, as the dark-adaptation in the alligator was complete within 5 to 7 minutes, whereas that in the frog took 30 minutes or longer. The rate of dark-adaptation was measured by recording the ERG of eyes first light-adaptated to a luminescence of 2000 mililamberts for 15 seconds and then kept in darkness for various time intervals. The authors point out that the rate of rhodopsin synthesis in the alligator eye resembles that of iodopsin synthesis in other animals. The higher rate of the latter, therefore, is not characteristic of the nature of the pigment.

Wald and Burg [101] investigated the nature of the vitamin A found in the eyes of the lobster (Homarus americanus). This investigation was stimulated by the surprising discovery of Fisher et al. (1952) that euphausiid vitamin A is only about half as potent biologically as an equal amount of alltrans-fish liver vitamin A. This suggested that the vitamin A in these animals is one of the less potent isomers of the alltransisomer. The experiments were carried out on Nova Scotia lobsters in December and January. The purple area containing the ommatidia of the eyes was cut off from the eye stalk so as to include all the pigmented tissue. A new procedure was developed which permits separation of the vitamin A from the astaxanthin present in large excess in the eyes and of the small amounts of β-carotene. The retinal tissue was dried with anhydrous sodium sulfate and extracted repeatedly with petroleum ether until the extracts were colorless. The astaxanthin was removed from these extracts by saponification. The total extract was dissolved in 6% KOH in methanol and incubated at 40 C for 20 minutes. The asthacene formed under these conditions from astaxanthin is removed by extraction with petroleum ether from the KOH methanol diluted to 60% with water. The washed petroleum ether is free of asthacene and contains β-carotene, the concentration of which was estimated from the extinction at 460 mμ in hexane. Vitamin A was determined in the nonsaponifiable fraction of the original petroleum ether extract, by the antimony chloride reaction. Its amount was found to be 1.18μg. per eye as compared with 3μg. of astaxanthin and 0.144μg. of β-carotene. The separation of the extracted vitamin A into the alcohol and ester fractions was carried out by the chromatographic procedure of Ganguly et al. (1953). It shows that 30% of the total was present as free alcohol. The absorption spectrum of vitamin A, the total amount of which was determined by the antimony chloride reaction, the intensity of which is identical for all isomers, was determined by a special newly developed procedure which makes it possible to obtain spectra of the vitamin initially present even in very crude extracts. The procedure is based on the determination of the absorption spectrum of the impurities contaminating the crude extract. extract with white light in presence of This can be done by isomerizing the crude catalytic amounts of iodine to an equilibrium mixture of isomers, determining the total amount of vitamin A, and subtracting the absorption due to these isomers calculated from the absorption of a standard solution of isomerized alltrans-vitamin A from the total absorption of the isomerized crude extracts. The absorption curve of the originally initially present vitamin A is then obtained as difference of the absorption curve of the crude extracts and that of the contaminants. In this way the authors could show a reasonable agreement between the initially present vitamin A and the neo-b vitamin A_1. The isosbestic point of the original curve and the curve obtained after isomerization was at 287 mμ, exactly

like that for neo-b vitamin A_1. Vitamin A was finally obtained from the retinas in pure form by chromatography of the petroleum ether extract of the 60% KOH methanol used for saponification of the crude extract on a column of calcium carbonate. Its optical characteristics were close to those of pure neo-b vitamin A_1. After oxidation to transretinene with manganese dioxide the absorption spectrum was again determined. The true spectrum of the retinene obtained in this way was determined by the same procedure as the spectrum of vitamin A and proved that the preparation consisted of 69% neo-b and 31% of transretinene. The authors suggest that some isomerization occurred in the course of the complex preparation procedures. This preparation, when added to bleached lobster rhodopsin, synthesized rhodopsin with a maximum of 498 mμ. After isomerization the absorption maximum was displaced to about 495 mμ, and only about one-quarter of the rhodopsin was formed as compared with the experiment with the nonisomerized preparation. The authors conclude that the lobster, by special mechanisms, is able to store in pure form in its eye a hindered form of vitamin A, which is in equilibrium with the alltrans form in a ratio of 95:5 in favor of the latter. They mention findings of other authors which indicate that neo-b vitamin A_1 is present also in other species of decapods and Eucarid crustaces.

Wald and Hubbard [102] determined the nature of the visual pigment of the lobster. The extraction was carried out on rhabdomeres, which resemble in their microstructure the outer segments of vertebrate rods and cones. The procedure was the same, therefore, as that used for vertebrate rods, based on fractionated centrifugation and floatation of the outer layers. The floating tissue sedimented after centrifugation in M/15 phosphate buffer pH 6.5 after 30 minutes centrifugation at 2000 ROH in the Spinco ultrapreparative centrifuge. The lyophilized sediment was extracted four times with petroleum ether until the extract appeared colorless. The sediment of this extract was again extracted in the cold with M/15 phosphate buffer pH 6.5, was washed again with distilled water until the supernatant was colorless, and was extracted overnight with 2% unbuffered digitonin. The solution was illuminated for one minute with orange light and then left in the dark with spectral recordings made periodically. The absorption at 490 mμ went down and the absorption at 370 mμ, marking the formation of retinene, increased. Difference spectra obtained in presence of hydroxylamine showed that the photosensitive pigment possesses an absorption maximum at about 550 mμ. The authors call it lobster rhodopsin. On irradiation, the extinction rise and the maximum is displaced to about 490 mμ. This intermediate resembles the so-called *m*-rhodopsin of vertebrates, which is apparently much more stable in lobster than in vertebrates, the latter remaining unchanged only at −20 C. The transformation of lobster *m*-rhodopsin into a mixture of opsin and retinene proceeds in the dark. $Retinene_1$ was identified by the absorption maximum of its opsin and of its reaction product with antimony chloride. This bleaching of lobster *m*-rhodopsin follows a course of a first-order reaction; at 29 C and pH 9.3 the time for half completion is 43 minutes. The reaction proceeds in a similar manner at pH 6.5.

Following up their determination of the vitamin A in the eyes of the lobster, Wald and Brown [103] also investigated the vitamin A from the eyes of a euphausiid crustacean. Twenty-four grams of eyes, representing several thousand animals, was extracted with petroleum ether containing 23% alcohol in a Waring Blendor. After evaporation, the oily extract was sealed under nitrogen and examined by the same procedure as used with the lobster material. The absorption curve of the crude extract displayed a broad maximum at 370 mμ, which moved to 324 mμ, on isomerization with light in presence of iodine, the E_{max} rising by a factor of 1.39. This indicated that the bulk of this material is neo-b vitamin A_1. The absorption of contaminating extrane-

ous material present in this crude extract was eliminated by the same procedure as was used in the case of the lobster. The absorption curves of the vitamin itself obtained in this way were those characteristic of neo-b vitamin A_1, and the same was valid for changes after isomerization and the position of the crossing point of the curves for unisomerized and isomerized vitamin A. The vitamin A from the extract was partly purified by chromatographic absorption of the petroleum ether extract of the saponified fraction obtained from the crude extract. Chromatography was carried out on a column of alumina weakened with 5% of its weight of water. The chromatogram was developed with 10% acetone in petroleum ether. The first filtrate, corresponding to half the total vitamin A, had an absorption maximum at 370 mμ, which after isomerization shifted to 325 mμ. E_{max} rose 1.38 times. The extinction of the subsidiary value at 324 mμ fell after isomerization to 0.56 of its former value, the factor for neo-b vitamin A_1 being 0.55. This purified vitamin A was oxidized with manganese dioxide. The isomerization product had an absorption maximum in hexane of 362 mμ, which after isomerization shifted to 367 mμ. After correction for extraneous absorption, the rise of E_{max} by isomerization was calculated between 1.58 and 1.64 mμ, that for neo-b retinene$_1$ being 1.7 mμ. From this, it is calculated that these preparations contained 84% and 92% of neo-b isomers, respectively. After addition of this preparation to cattle opsin in excess and two to three hours' incubation at room temperature, rhodopsin was formed in an amount which corresponded to 88% to 96% of that obtained with pure neo-b retinene; 90.7% of the vitamin was found to be present in ester form. The authors conclude that in this euphausiid crustacean the vitamin A in the eye is up to 90% present in form of the hindered neo-b isomer.

Barnholdt and Hjarde [104] isolated by chromatography five different vitamin A_1 isomers from the eyes of deep-water prawns (Pandalus vorealis). The tests used for the identification were isomerization with iodine as catalyst, formation of anhydro vitamin by means of HCl, determination of certain absorption parameters in the Carr-Price reaction, and the maleic anhydride reaction. Four of the isomers could be tentatively identified, while some indications were found that the fifth substance was the 9,13-di-cis vitamin A_1. As this investigation was carried out on the eyes of boiled prawns, the significance of these findings for the situation in vivo appears difficult to evaluate.

Yoshida [105] investigated the spectral sensitivity in the protozoan Diodema setosum. The dark-adapted excised parts of the aboral region of the intrambulaca were sensitive in the range of 440 to 610 mμ, with a maximum at 470 mμ. It agrees in this respect with the retinal pigment granules of frog and crayfish.

Blough [106] investigated the spectral sensitivity of the pigeon and found that the photopic and scotopic curves are similar to the absorption curve of iodopsin and rhodopsin.

Oyamada [107] studied the effect of L-methionine on retinal pigments in man and animals and found that it facilitates dark-adaptation, accelerates regeneration of the visual purple, decreases the electric threshold, and has no effect on fusion frequency.

Carli [108] reports a histochemical procedure for the demonstration of visual purple.

Matsumoto [109] found that tyrosine and tyramine accelerate the regeneration of visual purple in bull frogs.

Tani [110] reports an accelerating effect of thyroid and thyrotropic hormone on the regeneration of rhodopsin in the toad.

Ishiguro [111] investigated the creatine, phosphokinase, and phosphomonesterase of the retina. He obtained synthesis of ATP from creatine phosphate and AMP at pH 7 in dialyzed retinal extract of rabbit. At pH 8.4 the extract forms creatine phosphate from ATP. *p*-CMB inhibits these processes. Therefore, the enzyme is probably an SH enzyme. The author found two

monoesterases, one with an optimum at a pH of 4 to 5 and another at a pH of 9.

Inakuchi [112] found that the succinic and lactic dehydrogenase activities in dialyzed rabbit retina extracts are increased by Mg and decreased by Ca and *p*-CMB.

Sasaki [113] investigated retinal metabolism under the influence of stress and found that acute stress decreases O_2 consumption and aerobic glycolysis after three hours. After 24 hours the metabolism was increased, and it returned to normal after 48 hours.

Esposito [114] found that corticotropin (ACTH) inhibits O_2 consumption but not the aerobic glycolysis of rabbit retina in vitro and in vivo.

Auricchio [115] found that inflammatory conditions and resection of the optic nerve as well as I_2 administration did not change the potassium content of the retina, which, however, was decreased by monoiodoacetate.

Ezuka [116] found that dark-adaptation increases the ATP and creatine phosphate in the retina.

Nakazawa [117] found that fat substances in the outer limbs in vitamin A-deficient rats increased and decreased in the inner segments.

Dartnall [118] reviewed our present knowledge of visual pigments.

Sorsby and associates [118a] investigated whether degenerative changes in the retina first observed by Noell after intravenous injections of sodium iodoacetate can be also produced by other reagents which are able to bind thiolic groups. The dosage corresponded to about 80% of the maximum lethal dose in nearly every case. In spite of that, only bromoacetate, but not iodoacetamide, had an effect on the retina similar to that of iodoacetate. The thiolic reagents used represented heavy-metal derivatives, oxidizing agents, and alkylating agents. The authors suggest that the effect of iodoacetate is not due to its ability to combine with thiolic groups in the retina, as iodoacetamide, which is much less toxic than iodoacetate and as easily combines with SH groups, is completely ineffective.

Miscellaneous

Polatnick and associates [119] fractionated and analyzed mucopolysaccharides of the beef sclera by the fractionation procedure of K. Meyer and associates and compared it with the composition of the mucopolysaccharide of the cornea as reported in the literature. Two main fractions were obtained by fractionation with ethanol, one precipitating between 18% and 24% and the other, between 30% and 50% of ethanol. Further subfractionation within these limits of concentration did not yield any significant separations. The fractions were analyzed for nitrogen and hexosamine by the Elson-Morgan procedure, for sulfate by titration with $BaCl_2$ according to Alicino, for hexoses by the antrone reaction, and for hexuronic acid by the carbazole and orcinol reactions. The analytical data showed that the less-soluble fraction essentially is chondroitin sulfate B; the more soluble fraction or mixture of fractions seemed to be predominantly, on the basis of composition and rotation, a mixture of A and C, with an only insignificant admixture of hexose of 3.9%. The precipitation of the second fraction with ammonium sulfate and pyridine yielded no significant precipitate. The authors conclude, therefore, that no significant amounts of hyaluronic acid are present in the sclera. The total concentration of mucopolysaccharides was calculated to be between 0.7% and 0.9% of the dry weight, as compared with 2.2% found in the cornea; 50% to 56% of the fraction was represented by chondroitin sulfate B. The authors stress the absence of nonsulfated mucopolysaccharide in the sclera as a characteristic of this tissue as compared with all other so-far-investigated forms of connective tissue, and they relate the more closely controlled state of hydration of the cornea as compared with sclera to the absence of the nonsulfated mucopolysaccharide in the latter.

Dohlman [120] made a comprehensive study of the distribution of S^{35} in the rabbit eye after injection of labeled Na_2SO_4. The dis-

tribution of the labeled sulfate among five different parts of the globe and among three different chemical fractions of each ocular tissue was measured. The distribution among various parts of the eye was determined by autoradiography after injection of 20 mc. of carrier-free Na_2SO_4 intravenously into a 2.36 kg. rabbit. The animal was killed after 72 hours; the eye was fixed, immersed in benzene, and imbedded in paraffin. It was cut in 10μ sections. For the determination of the S^{35} in different parts of the globe, the latter was divided into five sections, namely, the cornea, sclera, lens, uvea, and the vitreous body plus retina. The eyes were enucleated 3, 17, and 43 days after injection of 1 mc. of Na_2SO_4 per kilogram intravenously. The distribution of S^{35} was determined in the inorganic phosphate, in the ester sulfate, and in the organic nonsulfate sulfur fraction. For this purpose the homogenized tissue was assayed for radioactivity; then, after addition of nonlabeled inorganic Na_2SO_4 it was dialyzed for four days against running tap water. The difference in radioactivity before and after dialysis was taken as an index of labeled inorganic sulfate and other dialyzable sulfates. The ester sulfate was determined in the dialyzed homogenate by hydrolyzing it for 24 hours in a boiling water bath with 6 N HCl, adding 40 mg. of nonlabeled Na_2SO_4 to each solution, and precipitating the sulfate as $BaSO_4$ with $BaCl_2$. The radioactivity was then measured in this filtered-off and dried precipitate. The excess barium in solution was then removed by addition of about an equivalent amount of sodium sulfate, and the supernatant was first dried down in vacuo over NaOH and then chromatographed on Dowex 2 Cl form, and the column was eluted with distilled water. This eluate contained the so-called nonsulfate sulfur. The amino acids in the eluate were then chromatographed according to the procedure of Stein and Moore on Dowex 50, and the sulfur-containing amino acids in this were separated from the others. The time-relation study was carried out by autoradiography after 1 hour, and 1, 3, 8, 17, and 31 days after administration of the isotope. Considerable incorporation was found already after one hour. It was, however, low in the lens and lower in the cornea than in the uvea and retina. After 31 days the radioactivity had disappeared from all parts except cornea and lens. After three days the aqueous and vitreous were free of S^{35}, indicating that there was no incorporation as organic sulfur. The cornea and sclera showed strong incorporation, the inner layers stronger than the outer parts. The epithelium contained some isotope, although it is not known to contain mucopolysaccharides. Lens, uvea, and retina showed much less incorporation than the two other parts of the eye. In the cornea 92% of the total S^{35} occurred as ester sulfate and 4.5% was found in the nonsulfate sulfur fraction. The latter later went up to 10% of the total S^{35}. Only 40% of the latter were present in S-containing amino acids; the rest, in a still-unidentified form. This nonsulfate organic S^{35} was predominantly in the lens and increased relatively and absolutely from the 3d to the 43d day. The uvea and retina contained the isotope in all three chemical fractions. The radioactivity in the epithelium, endothelium, and Descemet's membrane of the cornea was determined separately and was found to represent after 3 days 4% and after 43 days 6% of the total radioactivity of the cornea.

Heydenreich [121] carried out a thorough investigation of pigment granules found in all parts of the eye. In addition to morphological findings, he also reports biochemically interesting results on various bleaching procedures for the pigments and their ability to combine with dyes. The most effective procedure for bleaching was found to be the treatment with oxidizing agents. Ten per cent sodium peroxide and ten per cent ammonium persulfate were most effective as bleaching agents, and the first proved superior to hydrogen peroxide. The fastest procedure proved to be direct exposure to chlorine vapors and treatment with 2% to 3% chromic acid. The pigments

from different parts of the eye showed a very different stability against the bleaching agents. Dyeing of the pigment granules was most effective after bleaching before complete fragmentation of the bleached granules. Only basic dyes, however, were taken up by the granules. Pretreatment, however, with alkaline reagent could change the dyeing properties, and the author explains the discrepancies between his findings and those of other authors by differences in the pretreatment of the granules before exposing them to dyes. The impregnation with silver was only effective in the nonbleached pigments, and in partly bleached pigments, it could be shown that the core of the pigment granules is much more labile to impregnation than the periphery, while the opposite was found to be true with dyes. The results of the author do not suggest essential differences in the chemical nature of pigments of different origin, assumed by other authors.

Dardenne and associates [122] investigated whether the cholinesterase which was previously found to be present in the iris is the so-called specific cholinesterase present in nerve tissue and red cells or the nonspecific cholinesterase present in the blood plasma. This appeared of interest because of the different behavior of the two enzymes against drugs used as miotics. To distinguish between the two different cholinesterases several criteria were used: (1) the dependence of the enzymatic activity on the concentration of the substrate, (2) differences in the activity towards various types of substrates, and (3) the sensitivity of the enzyme against coffein. The cattle iris was used five hours after the death of the animals, during which time it was stored at 2 C. Human iris was obtained from eyes enucleated 8 to 15 hours after death; 30 to 100 mg. of wet iris tissue was used for every experiment, and the rate of the splitting of acetylcholine was measured by the Warburg method in a Ringer bicarbonate solution in an atmosphere of 95% N_2 plus 5% CO_2. The concentration rate curve of the iris enzyme showed the bell-shaped form characteristic for the specific cholinesterase. The homogenate of the iris split easily acetyl β-methyl choline and did not split benzoylcholine at all, and coffein at a concentration of 3.5×10^{-2} inhibited the enzyme by more than 80%. The authors point out the possible significance of these findings for the treatment of glaucoma with cholinesterase inhibitors.

Cibis and associates [123] examined the effect of intravenous injections of iodoacetate on the dynamics of the aqueous and the lens and compared it with the effects of x-rays. This investigation was stimulated by the parallelism previously found by Noell on the similarity of the effect of x-rays and iodoacetate on the degeneration of the retina. Iodoacetate, 20 mg. per kilogram, was injected intravenously into male albino rabbits. A second injection followed 6 hours later, and in some animals a third injection was given after 24 hours. Control animals were killed at the same time as the experimental animals. In 16 rabbits the acute effects of IAA upon the blood-aqueous barrier were followed with the slit lamp over a period of 13 days. Appearance of cells in the anterior chamber was observed the second or third day after the injection, reached its maximum on the fifth day, and later declined. The majority of the cells were erythrocytes, mixed with a few lymphocytes and polymorphonuclear leukocytes. The effects upon the rate of secretion of aqueous were studied by repeated tonographic tracings. After injection of 40 mg. of IAA per kilogram, the rate of aqueous secretion was calculated from the intraocular pressure and facility of outflow, assuming constant episcleral venous pressure of 9 mm. Hg. The rate of aqueous flow decreased 48 hours after injection and reached a minimum at 72 hours. Recovery was complete after five to seven days. The effects on the mitotic activity of the lens epithelium were studied on 41 experimental eyes, and the separation of the lens epithelium was made according to the method of von Sallmann. During the first 24 hours the mitotic activity was not impaired, but

it was found to be inhibited in approximately 60% of the cases between the 2nd and 12th days. The recovery was found after 16 days. The authors also report late lenticular changes induced by IAA. They find that the changes induced by IAA and ionizing radiation correlate well, in spite of certain differences. These differences manifest themselves in the nature of the cells in the cameral exudate and in the time sequence of mitotic inhibition and suppression of aqueous flow. The authors stress, however, that at least one part of these differences might be caused by differences in the routes of application, which is systemic in the one and local in the other case.

Ito [124] found that iris extracts of rabbit oxidize pyruvate, oxaloacetate, citric, malic, and succinic acids. Activities of these enzymes were promoted by ATP and Mg as well as by cocarboxylase and were inhibited by HCM.

Fukazawa [125] found that O_2 consumption of the chicken iris decreases after three weeks of thiamine deficiency.

Hama [126] investigated the distribution of β-glucuronidase in ocular tissues. A high concentration was found, particularly in the ciliary body. Its activity was decreased by phenol and menthol administration. Naphthaline administration caused considerable variations in the activity of this enzyme in the ciliary body, iris, and choroid.

Bernardinis and Gaipa [127] found with histochemical methods that iodoacetate decreases the level of RNA in the ciliary body, retina, and uvea of rabbits after 3 to 18 hours. DNA increases after five days.

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News and Comment

GENERAL NEWS

Section on Ophthalmology, American Medical Association.—The Annual Scientific Session of the Section on Ophthalmology, American Medical Association, was held in San Francisco from June 24 to 26, 1958. Approximately 400 persons, a record for attendance on the West Coast, were present throughout the meeting. Prof. Gosta Karpe, of Stockholm, Sweden, was the Guest of Honor of the Section.

The following prizes and honors were awarded:

1. The Prize Medal in Ophthalmology was awarded to Peter C. Kronfeld for his many contributions to our specialty.

2. The $250 prize for the best exhibit was awarded to L. K. Garron, M. J. Hogan, W. K. McEwen, and M. L. Feeney for their exhibit, "Electron Microscopy of Ocular Tissue."

3. The $250 prize was awarded to Warren A. Wilson for his paper, "Galactosemia with Associated Cataracts in Children," judged on the basis of presentation and originality.

The following officers were elected: Frank B. Walsh, Chairman; Philip M. Lewis, Vice-Chairman; Henry F. Allen, Assistant Secretary; Ralph O. Rychener, Delegate to House of Delegates; W. Howard Morrison, Alternate Delegate to House of Delegates; Frank W. Newell, Representative to Scientific Exhibit.

PERSONAL

Dr. John R. Fair.—Dr. John R. Fair has been promoted to be Associate Professor of Surgery (Ophthalmology) at the Medical College of Georgia, Augusta.

SOCIETY NEWS

American Ophthalmological Society.—At its recent meeting, the American Ophthalmological Society elected the following officers for the coming year: President, Dr. Derrick Vail, Chicago; Vice-President, Dr. Algernon B. Reese, New York; Secretary-Treasurer, Dr. Maynard C. Wheeler, New York; Editor, Dr. Gordon M. Bruce, New York.

ANNOUNCEMENTS

Seminar on Glaucoma.—A seminar on glaucoma with particular emphasis on gonioscopy and the study of the anterior angle will be given at the Brooklyn Eye and Ear Hospital on Nov. 17, 18, and 19, 1958. Ample opportunity for practical instruction in the use of the gonioprism will be given, and material from the glaucoma clinic will be utilized.

The Course will be given by Drs. Daniel Kravitz, assisted by Drs. Mortimer A. Lasky, A. Benedict Rizzuti, Abner S. Rosenberg, Nicholas P. Tantillo, and Samuel Zane.

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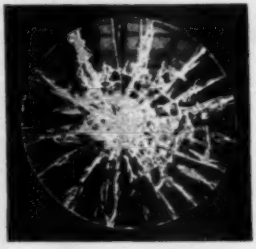

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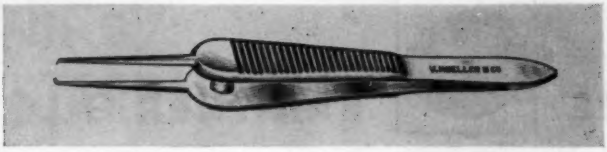

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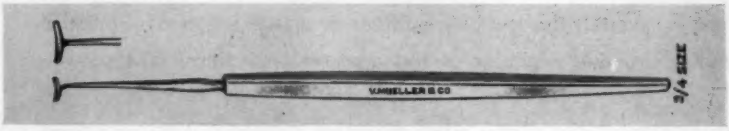

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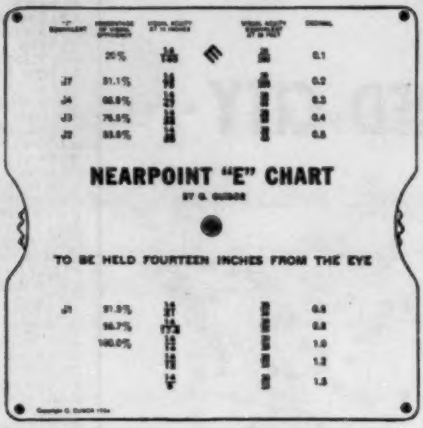

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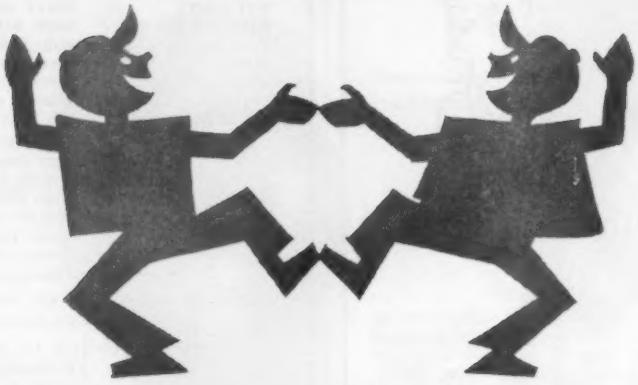

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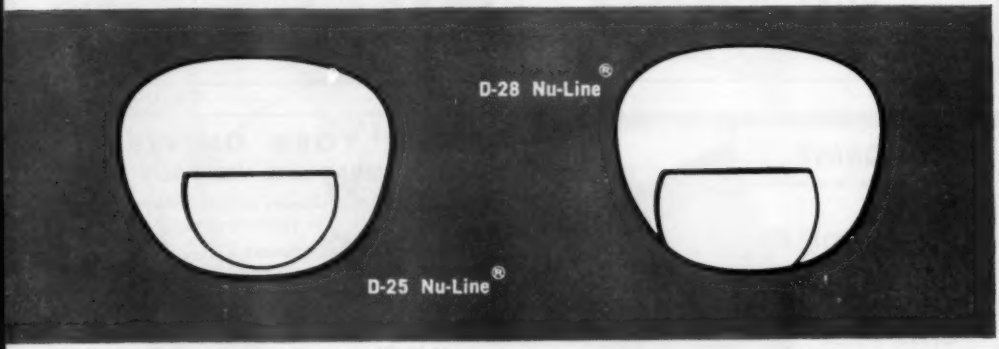

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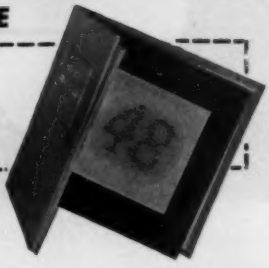

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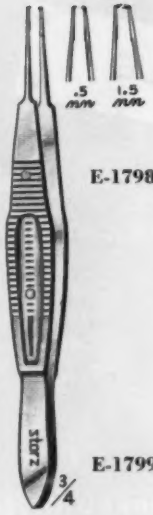

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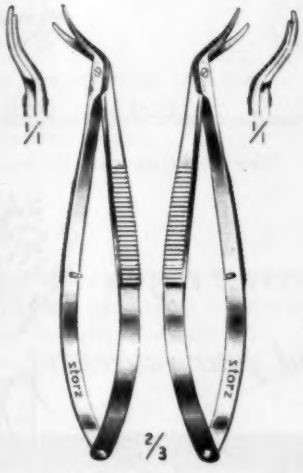

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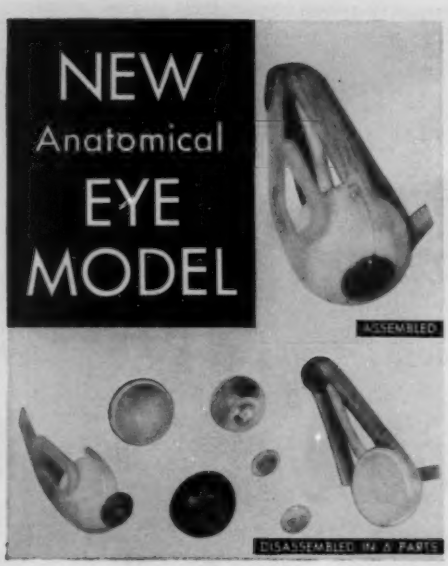

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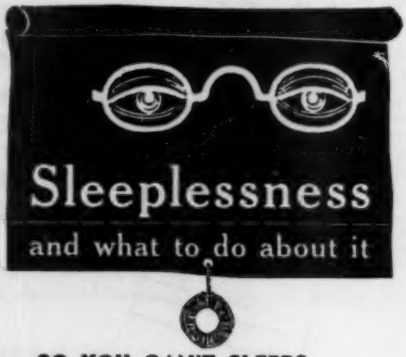

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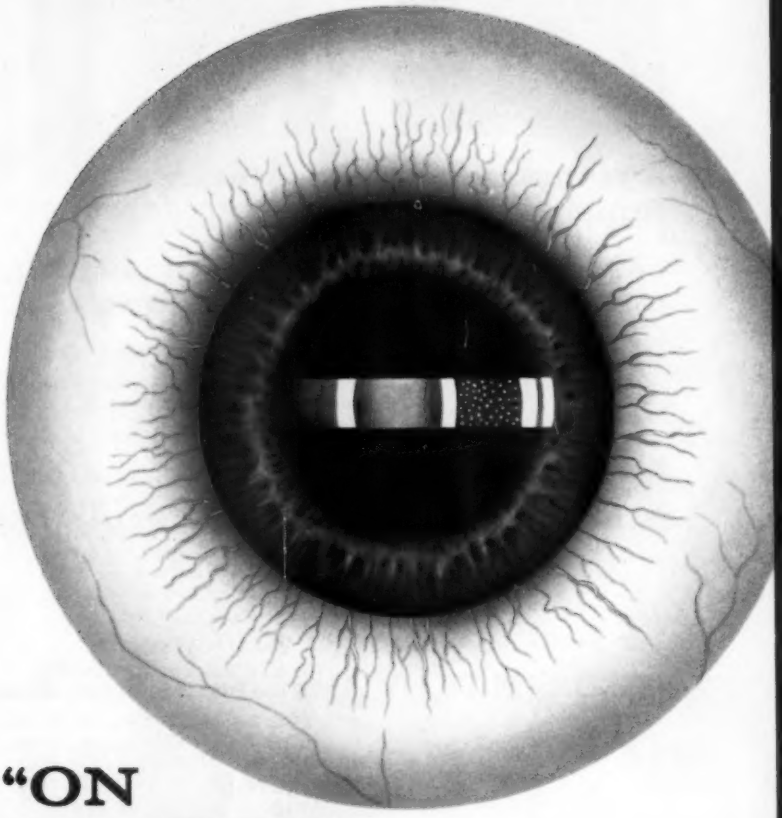

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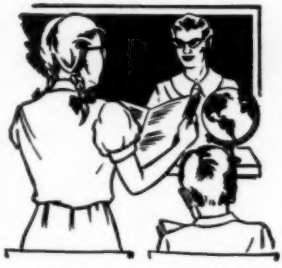

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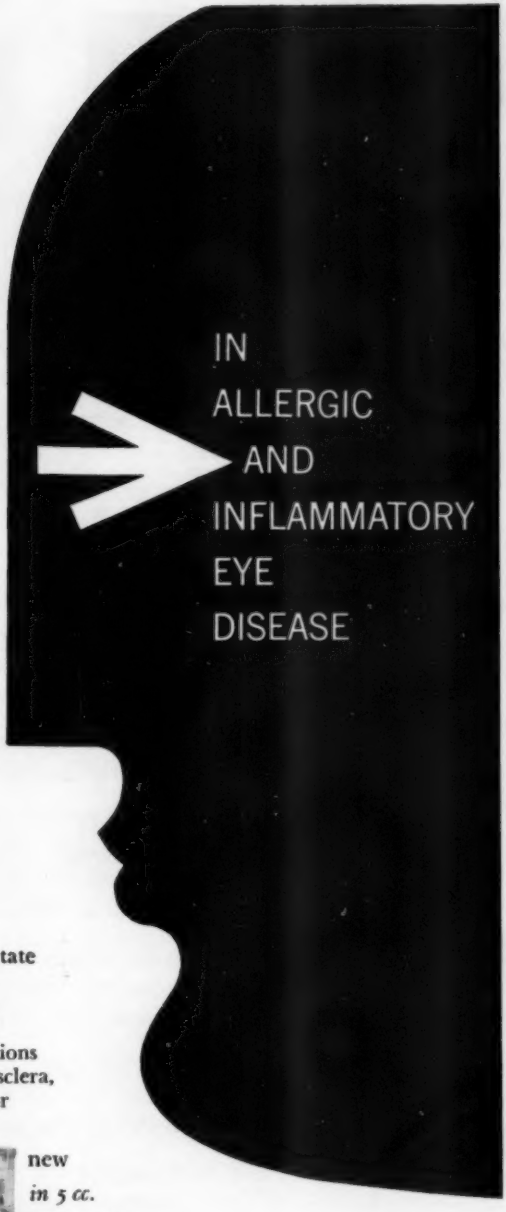

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